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The Journal of Laboratory and Clinical Medicine

VOL. XII ST. LOUIS, MO., MAY, 1927 NO. 8

CLINICAL AND EXPERIMENTAL

THE EFFECT OF VENOUS STASIS ON THE PROTEINS OF BLOOD PLASMA AND ON THE RATE OF SEDIMENTATION OF THE RED BLOOD CORPUSCLES*

BY E. D. PLASS, M.D., AND M. D. ROURKE, M.S., DETROIT, MICH.

IT HAS been well established that venous stasis leads to dehydration of the blood. Dautrebande, Davies, and Meakins[1] have shown that such a procedure causes passage of both electrolytes and water to the tissues, while Grawitz[2] and Schultz and Wagner[3] have demonstrated marked increases in the erythrocyte count, the hemoglobin content, and the specific gravity of the whole blood. A rise in total plasma protein percentage during venous stasis has been proved by Kreibich,[4] Rowe,[5] Peters, Bulger, Eisenman, and Lee,[6] Böhme,[7] and others. Rowe's[5] studies on the increases of the albumin and globulin fractions of human blood serum in eleven pathologic cases with as many different diagnoses showed that in general the percentage increase of albumin was higher than that of the globulin, although three cases show the reverse.† Rowe also pointed out that the protein increase is a function of the duration of the stasis, and that a stasis as short as one and one-half minutes increased the albumin by 5.65 per cent and the globulin by 3.71 per cent. Peters, Eisenman, and Bulger,[8] in 1925, reported two experiments on venous stasis where they studied simultaneous changes in total plasma protein percentage and plasma volume percentage, and found the plasma protein increase of the same order of magnitude as the plasma volume decrease. They explain their results on the theory of simple plasma concentration with no transfer of protein to or from the tissues. They disregard, however, the very marked differences between the percentage increases of the albumin and globulin fractions pointed out by Rowe[5] and amounting to from 27 to 58 per

*From the Obstetrical Department of the Henry Ford Hospital.

†Rowe used Robertson's refractometric method.

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cent in seven of his eleven cases. Moreover, in two out of three later experiments reported by Peters, Eisenman, Bulger, and Lee,[6] they found a plasma volume decrease of 14.8 per cent with a total protein increase of 36.0 per cent in one instance, and in the other a plasma volume decrease of 4.9 per cent with a total protein increase of 18.5 per cent.* From these last two experiments and those of Rowe, it is evident that the plasma protein increase cannot always be explained by a simple concentration hypothesis.

We could find no mention in the literature of the effect of stasis upon the sedimentation rate of the red blood cells, although it might have been assumed from the work of Fahraeus[18] that the rate would be diminished by reason of the increased number of red cells which had previously been described in venous stasis.

The experiments here reported show the effects of venous stasis of varied duration on the fibrin, globulin, and albumin contents of the plasmas of normal individuals, together with the plasma volume percentages and the sedimentation rates of the red blood corpuscles. These simultaneous experiments were designed to bring out more clearly the relation of plasma volume to protein increase during venous stasis and so lead to a more rational theory of the mechanism of this increase.

EXPERIMENTAL

The subjects rested in bed for twenty minutes with the hands immersed in warm water, after which a tourniquet or the arm band of a blood-pressure apparatus was applied to the left arm above the elbow. The pressure applied with the tourniquet was sufficient to cut off the venous return without obliterating the radial pulse. When the blood-pressure apparatus was employed, the pressure was maintained constantly at a point midway between the systolic and diastolic pressures. In most instances the samples were drawn about three and one-half hours after breakfast. In every case the arm showed marked cyanosis when the blood was drawn and when the pressure was applied for fifteen minutes or more numerous petechial areas extending from the margin of the constricting band to a point several inches down the forearm were observed. These latter subjects also complained of tingling and numbness of the fingers. All blood was collected directly under oil in paraffined 15 c.c. centrifuge tubes. The bloods used for the sedimentation rates were taken over 0.04 c.c. of heparin solution containing 6.0 mgm. of heparin for reasons to be discussed in a later communication, while those used for plasma volume percentages and plasma proteins were collected over 0.07 c.c. of a saturated solution of potassium oxalate for each 14 c.c. of blood.

Fibrin, globulin, and albumin determinations were made by the colorimetric method of Wu.[9]† Analyses were done in duplicate and excellent checks were obtained. Plasma volume percentages were determined in 15 c.c. centrifuge tubes rotated for twenty minutes at 2500 R.P.M.

*The third case shows a plasma volume decrease of 18.3 per cent with a total protein increase of 20.9 per cent.

†The albumin was calculated from the formula $\frac{20}{Ra} \times 5.50$. The factor 5.44 in the condensed formula as reported is evidently a misprint.

Sedimentation rates were determined upon 1 c.c. samples of heparinated blood in paraffined tubes of 3.86 mm. in diameter which gave a column of blood 85 mm. high. These tubes are somewhat similar to those described by Linzenmeier.[10] The supernatant layers of clear plasma were measured at frequent intervals with an accurate millimeter scale. All determinations were done at room temperature and the samples kept under oil, the blood having been

Case No.	Arm-band pressure	Duration of stasis	Plasma volume			Fibrin			Albumin			Globulin			Total protein			Remarks
			No stasis	Stasis	Dec.	No stasis	Stasis	Inc.	No stasis	Stasis	Inc.	No stasis	Stasis	Inc.	No stasis	Stasis	Inc.	
	mm.	min.	%	%	%			%			%			%			%	
1 P.J. ♂	90	5	55.3	51.8	6.3	0.30	0.34	11.7	4.80	5.24	9.2	2.53	3.02	19.4	7.63	8.60	12.7	Arm cyanotic. No pain.
2 R.S.S. ♂	Tourniquet	8	59.7	57.7	3.6	0.24	0.27	12.5	4.77	5.13	7.5	2.21	2.30	4.1	7.22	7.70	6.6	Arm cyanotic. No pain.
3 E.D.P. ♂	Tourniquet	12	57.4	57.9	** 0.9	0.35	0.38	7.1	5.00	5.47	9.4	2.16	2.40	10.1	7.53	8.25	9.6	Arm cyanotic. Slight tingling. Arrested tuberculosis explains the high fibrin.
4 M.J. ♀	90	15	62.3	56.9	8.7	0.29	0.38	31.2	5.02	5.79	15.3	2.27	2.70	18.9	7.58	8.87	17.0	Petechiae on arm. Two weeks after menstruation
5 C.W.M. ♂	90	15	55.1	46.9	14.9	0.26	0.34	31.0	4.70	6.09	29.6	2.16	2.60	20.4	7.12	9.03	26.8	Petechiae
6 H.C.W. ♂	Tourniquet	16	58.7	55.8	4.9	0.23	0.25	6.4	4.64	5.53	19.2	1.99	2.31	16.1	6.86	8.09	18.0	Arm cyanotic.
7 M.D.R. ♀	100	20	62.3	53.5	14.1	0.24	0.33	39.0	4.85	6.88	41.9	2.50	2.58	3.2	7.59	9.79	29.0	Pain, tingling, and petechiae. Tetany of hand after release of pressure. Fourth day after onset of menstruation.
8 M.D.R. ♀	100	20	61.1	45.0	26.4	0.29	0.45	58.0	4.76	7.70	61.8	2.47	3.47	40.5	7.52	11.62	54.5	Pain, numbness, and petechiae. Tetany of hand after release of pressure. Fourteen days after onset of menstruation.
9 D.G.C. ♂	90	21	55.6	48.1	13.5	0.29	0.37	23.4	5.00	6.76	35.2	2.31	3.57	54.5	7.60	10.70	40.8	Marked petechiae. Total plasma solids increased from 8.55 to 12.38 percent—(increase of 42.5 %)
10 M.R.W. ♂	100	22	57.6	44.3	23.4	—	—	—	4.45	6.48	45.6	2.27	3.10	36.5	* 6.72	* 9.58	* 42.6	Tingling, numbness and numerous petechiae.
11 E.A.T. ♀	80	26	65.0	62.0	4.6	—	—	—	5.25	5.66	7.8	2.51	2.94	17.1	* 7.76	* 8.60	* 10.8	Tingling and petechiae. Fourth day of menstruation.

* Serum protein ** Increase

Table I.—Showing the effect of venous stasis upon the plasma volume and upon the concentrations of the various plasma proteins.

transferred from the original centrifuge tube by means of a micropipette and never allowed contact with the air. (This method will be published in more detail shortly.)

Table I presents our plasma protein results in detail.

DISCUSSION

Our results showing plasma volume decreases due to loss of fluid to the tissues during venous stasis confirm those of Peters and his coworkers,[6] Grawitz,[2] and others. The increases in the globulin and albumin fractions generally confirm Rowe's[5] figures. Six of the eleven cases show the globulin to have increased to an appreciably greater extent than the albumin. The duration of stasis, as Rowe[5] pointed out, is a controlling factor in the magnitude of the changes brought about, but the amount of pressure applied and the personal

element also have a part. Application of the simple tourniquet usually did not produce so great a change as the pressure controlled by the arm band of the sphygmomanometer. The pressure exerted by the former undoubtedly varies considerably since there is no true method of control within wide limits.

In no case of the eleven reported is the plasma volume percentage decrease even substantially equal to the total protein increase or equal to the fibrin, globulin, or albumin increase when considered alone. The change in plasma volume is due almost entirely to loss of fluid to the tissues as is shown in Experiment No. 9, where the cell volume increase was found to be 16.9 per cent and the cell count increase 18.2 per cent. The increase of total protein or of any particular protein cannot, therefore, be ascribed to a simple loss of fluid to the tissues. That no appreciable increase of the protein content of the plasma could be effected by synthesis of the amino acids and peptid nitrogen is shown by the constancy of the nonprotein nitrogen figures during stasis reported by Rowe and confirmed by us in Cases No. 9 and No. 10, where the nonprotein nitrogen rose from 39.2 to 50.0 mgm. per 100 c.c., and from 30.8 to 36.1 mgm. per 100 c.c. respectively. This apparent rise can be explained by the increased cell numbers, cells being higher in nonprotein nitrogen than the plasma. The initial high value in the first instance may be due to the fact that the specimens were taken about one hour after breakfast. That the increase in plasma protein cannot be accounted for by a decrease of corpuscle protein is shown by experiments on Cases No. 9 and No. 10, in which the corpuscle protein changed only from 38.5 to 38.6 grams per 100 c.c., and from 36.3 to 34.7 grams per 100 c.c., respectively, due to stasis. The whole-blood protein was determined by the Kjeldahl method and the corpuscle protein calculated from these results together with the plasma protein and the cell-volume figures.

Starling[11] has shown that normally the lymph in the extremities contains only from 2 to 4 per cent protein, the lymph in the intestines from 4 to 6 per cent protein, and that in the liver from 6 to 8 per cent protein. From these facts and a consideration of Traube's theory of membrane permeability we might conclude that the sieve structure of the capillary walls of the liver is coarser than the structure in the extremities and allows the large hydrated solute molecules, the proteins, to pass more easily through the interstices. In certain pathologic conditions, wound shock,[12] hemorrhagic shock, histamine shock,[13] and others, it has been shown conclusively that the capillary walls in the extremities have become much more permeable to protein and that the formation of lymph has increased, thus dehydrating the blood. Heidenhain[14] has shown that peptone is a powerful lymphogogue and that the lymph formed may be richer in protein than the blood plasma.

The tissues of the arm during stasis suffer from acute anoxemia. Under this condition, fixed acid products are formed within the tissue cells as soon as the process of oxidation is handicapped (Koehler, Brunquist, and Loevenhart[15]). The carbon-dioxide content must increase continually both in the tissues and the blood in the absence of a means for its removal or of a sufficient supply of reserve alkali. Consequently, there has been induced a tissue and blood

acidosis.[6] That this critical state of anoxemia and acidosis in the tissues has probably led to the formation of products which affect the physical-chemical properties of the membranes, increasing their permeability, and which bring about tissue hydration, is shown not only by the sudden local change of plasma protein in our experiments and those of Rowe et al., but also by a very conclusive experiment of Mann.[16] This latter observer produced venous stasis in the four extremities of a dog for an extended period and observed that the animal went into shock when the tourniquets were removed, allowing the products which had accumulated in the extremities to circulate throughout the system.

As a possible explanation for the increased protein content of the plasma we suggest that products resulting from the anoxemia have altered the permeability of the cell membranes, allowing protein from the cytoplasm, admittedly high in protein, to diffuse to the lymph and thence to the blood through the capillary walls, to aid in preserving the osmotic relationships which have been disturbed by the changed physical and chemical conditions.

The prompt and large increase in the plasma fibrin during venous stasis is a very interesting observation. The fibrin increase is local to the arm from which the blood flow is blocked and cannot, therefore, be attributed to any specific liver stimulation. This observation would seem to demand that there be reserve fibrin in the tissues of the arm as has been suggested by Foster and Whipple[17] and others.

SEDIMENTATION RATES

The sedimentation rates of the red blood cells increased during stasis in all cases except No. 2 where the total protein rise is smallest and where there appears to be an abnormal suspension stability without stasis. Fahraeus[18] has shown that the rate of sedimentation is influenced by the corpuscle number, all other factors being constant, and that, the higher the corpuscle number, the slower the rate of sedimentation. He also showed that the sinking velocity is about doubled by a reduction of the corpuscle number from 5,000,000 to 4,000,000 per cubic millimeter. Stasis increases the corpuscle number, which effect would tend to decrease the rate of sedimentation, barring other changes in the blood. Therefore, the difference between any two curves representing the sedimentation rates with and without stasis would be even greater were the corpuscle number the same in the two samples.

The same general type of curve is followed in most cases. The rise during the first few minutes is less sharp, as is characteristic of the period of primary agglutination, and is followed first by the period of sedimentation which is not impeded by packing and then by the portion showing the damping of the rise due to packing of the corpuscles. In practically every case, the curve representing the stasis rate crosses or tends to cross the curve representing the normal rate as they both approach their limits of sedimentation. Since the hematocrit reading is higher for the blood collected during stasis, the limit of plasma height is lower and therefore the curve representing the stasis rate must cross the normal.

We are not able to say definitely which of the variable factors brings about an increase in the rate of sedimentation during stasis.

The nature of the physicochemical properties of the superficies of the micellae of any colloidal system is dependent upon the properties of the medium in which the particles are suspended. In turn, the stability of a colloidal system is dependent upon the properties of the superficies. We should, therefore, look for changes in the suspension medium—the plasma—which can affect the physicochemical properties of the superficies of the

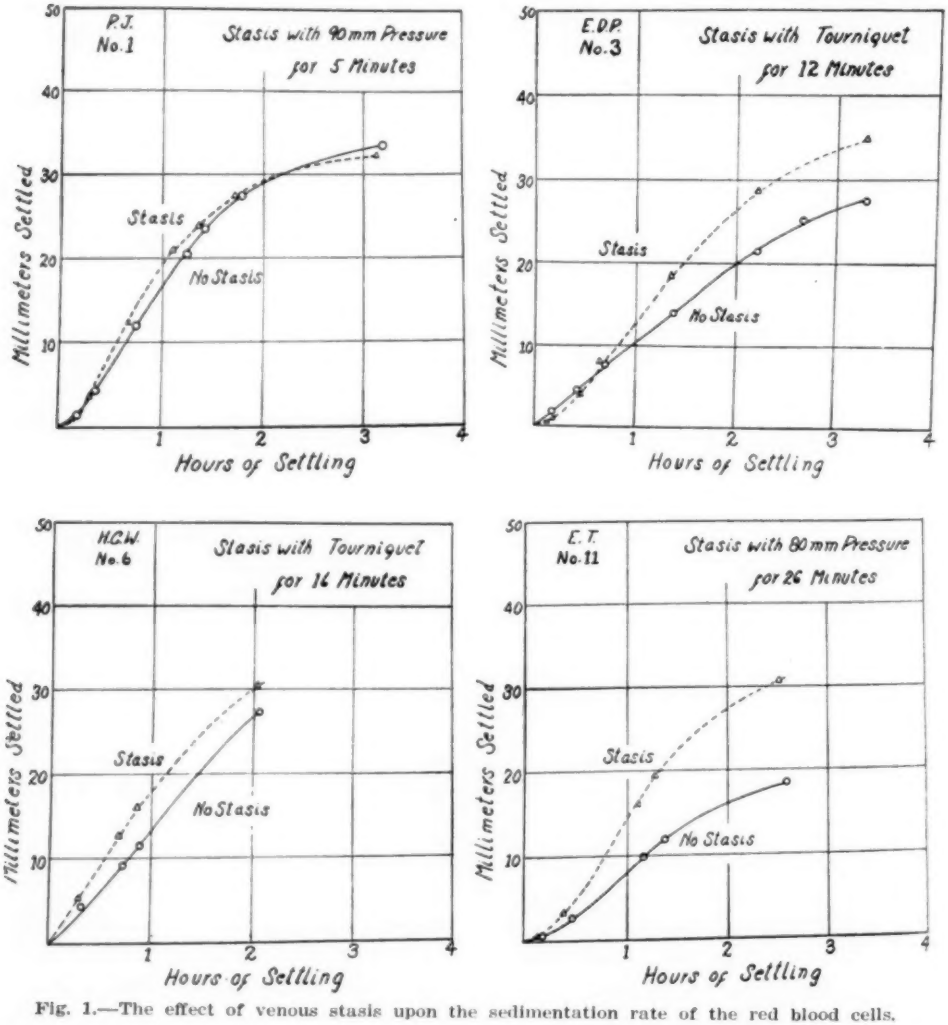

Fig. 1.—The effect of venous stasis upon the sedimentation rate of the red blood cells.

micellae—the red blood corpuscles—and can alter the stability of the blood as a colloidal system.

The P_H of the plasma has been reported by Peters[6] to be appreciably lowered by venous stasis, but the effect of different hydrogen-ion concentrations on the sedimentation rate of the red blood corpuscles has not yet been studied. Plasma chlorides, as reported by Peters[6] and confirmed by our experiments, are somewhat lowered. Fahraeus[18] has shown that the addition of sodium chloride decreases the rate of sedimentation. The magnitude of

the decrease of sodium chloride, which is produced by stasis, is, however, very much less than that by which Fahraeus produced his results, and it can hardly be imagined that it could effect changes of the magnitude we have demonstrated. Plasma oxygen and carbon dioxide tensions are also changed by stasis, but the effect of these changes on the sedimentation rate has not yet been studied carefully. Increased corpuscle number has been shown to produce a decreased rate of settling, the opposite of the effect we noted from stasis.

Fahraeus[18] and many others have pointed out the important rôle of the plasma proteins in determining the rate of settling of the red blood cells but there is considerable difference of opinion as to which protein is concerned. Fahraeus feels that an increased sedimentation rate is usually explained by a globulin increase, while Linzenmeier[19] attributes the variations to what he calls the "Senkungsbeschleuniginde Substanz" and which he identifies more or less positively as fibrin. Musa,[19] Westergren,[20] Gram,[21] Starlinger,[22] and many others attribute an increased rate of settling in certain conditions very definitely to increased fibrin, and we prefer to assume this position, not losing sight of the fact that some of the other factors which have been mentioned may have more or less influence.

SUMMARY AND CONCLUSIONS

Prolonged venous stasis leads to blood dehydration and to an increase of plasma proteins. The fibrin, globulin, and albumin are each increased and to a greater extent than can possibly be accounted for by concentration of the plasma alone. The increase in the separate proteins are not equal to each other, nor is any one consistently higher or lower than the others.

We believe that certain catabolic products formed in the tissues by reason of the anoxemia imposed by the venous stasis have acted upon the cell "membranes" of both the capillaries and the tissue cells, increasing their permeability to protein.

It is suggested that the lymph fluid has increased in protein content by diffusion of protein from the cytoplasm of the tissues which it surrounds, and that this lymph fluid, having become higher in protein than normal plasma, subsequently loses protein to the plasma, thereby increasing the total plasma proteins.

The marked increase in fibrin during venous stasis points to reserve fibrin in the tissues.

The sedimentation rate is increased by prolonged venous stasis and it is suggested that the increase is probably due chiefly to increased fibrin.

Care should be exercised in the use of the tourniquet or other constriction during the taking of blood upon which the sedimentation rate or plasma proteins are to be determined.

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THE PATHOGENICITY OF THE SMALL RACES OF THE "AMEBA OF DYSENTERY"*

By Rawson J. Pickard, M.D., San Diego, California

CONJOINT life is a test of strength between its participants. The relationship is seldom a true symbiosis, of benefit to both; practically always one party is the giver, and even when he gives from excess he must always be on guard lest his partner, flourishing, does not take from his need. Each organism strives for the full development of its potentialities. A change in environment changes their relative strength, so that from a benign commensalism, in which the lesser lives upon the waste of the greater, they may pass into a parasitism causing the disease or death of one of the participants. Thus a parasite ignored in its weakness may attack from strength, or from numbers, when conditions favor it. So, as with any form of life, the balance is not stable; changing surroundings release or inhibit inherent capacities of expression which bring about physiologic changes, often accompanied by changes in morphology. With two different species in some form of conjoint life the balance in their relationship is the more easily disturbed in that the advantages to be seized by one or the other are multiplied by the differences in capacity of expression between different kinds of organisms. And peace is never declared in nature.

In discussing the relationship between man and the ameba, or other intestinal protozoa, we properly use in our terminology such terms as variable virulence, immunity, and so on, from the partisan point of view of mankind; but in studying these organisms we avoid prejudicing a true estimate of the status of any parasite by the careless use of words which are applicable in an opposite sense from the parasitic standpoint.

The difficulties in the study of human intestinal protozoa are great. The careful study of parasitic protozoa in man is only a few years old. Few of these protozoa have been cultivated at all, the ameba of dysentery only recently and then in association with bacteria. The protozoa are not only more difficult to study than the bacteria, but they are also more difficult to recognize. Their more complex forms are often degenerated, dying or dead, when discovered, and sufficiently resemble other cells of various kinds to require considerably more specialization to recognize than is required for a bacterial diagnosis. Clinically the difficulties are even greater. "We have been highly trained in thinking from the bacterial point of view since the days of Pasteur, and poorly trained in thinking from the point of view of protozoon infection," says Kofoid.[1] We are carrying over into the domain of parasitic protozoology ideas that belong to bacteriology. With bacterial infection are the classic rubor, dolor, tumor, calor, and characteristic changes in the blood. Haugh-

*Received for publication, January 18, 1927.

wout,[2] in an early paper on the pathogenicity of the flagellates, speaks of the remarkable powers of adaptation shown by both the free living and parasitic protozoa* and asks, "Does it not seem that we are dealing with chemical affinities of a different nature, chemical reactions governing the regulation of diseases of a characteristically nonfebrile character unaccompanied by the phenomena of immunity? These phenomena need not be restricted necessarily to the metabolic chemistry of the parasite. They might be influenced by chemical changes originating in the cells and body fluid of the host." Haughwout was the first to perceive that the protozoan diseases are different in nature from the bacterial diseases, and require special methods of study,—methods perhaps only in the devising at present.

Several of the species of human entamebae are regarded as always remaining as harmless commensals, the flagellates sometimes seem to be so, and the ameba of dysentery, now found to be a common inhabitant of the human intestine, while amebic dysentery is rare—in the temperate zone,—has had its status again brought in question. Yet with any parasite, however tolerant the association may appear as deduced from the crude tests we are able to apply in our laboratories, there exists a complex counterreaction between the host and the parasite, the complexity increasing with the differentiation of the organs of the associates. At one extreme the protozoan is quite definitely and evidently harming its human host; at the other extreme the protozoan is not only well enough tolerated that no signs of damage can be discerned, but, on the basis of encystment, might be said to be on the defensive. The summary dismissal of species which are probably not pathogenic, as harmless, however, is not a "scientific" attitude. While it would doubtless be wrong not to admire the hesitation of the zoologists to condemn a human parasite in the absence of complete proof of its noxiousness, they judging for medical matters as they properly judge in other biologic questions, yet it must be remembered in favor of physicians, when they look with mistrust on all parasites, that medicine, like war, is primarily an art that utilizes science—an art with a necessary admixture still of rite (again like war)—and the clinician, in the presence of a sufferer, feels that the individual cannot wait for the slow tide of absolute conviction to arise in the scientific sea, for the drowning of his ills. The clinician may well let an empiricism be his guide until a better one be found, and use on insight like that which led Goethe to a divination of the substance of evolution. A working hypothesis based on the available facts is of more use to the sick man than to wait for missing links which may unite the facts and theory into the force of a law. It is necessary of course for the physician to remember that he is working on a theoretic basis, and that he be ready to discard it for one better substantiated, nor must he let himself think that one case recovered or cured is proof for the moment's fashionable idea.

We are at present sufficiently informed to say that parasite, and not commensal, is the term which properly describes the simpler organisms which

*Some interesting instances of protozoan adaptability are given in a paper by Strong on "The development of pathogenicity" in the "International Conference on Health Problems in Tropical America," 1924, United Fruit Co., Boston.

live in more complex forms. Their secreta and excreta are certainly unfitted for absorption and use by the higher organism, since the protozoan, dependent on the host for its food, must utilize this food according to its own metabolic processes, which are unquestionably different from those of the host. Therefore, during the course of an infection, there must be produced products which if absorbed by the host will affect his welfare in proportion to the delicacy and sensitiveness of the reaction of his body, largely a function of his body complexity and especially that of the neuropsychic apparatus. These are in addition to any products that might arise from a disease affecting the parasite itself, such as immune bodies against the host, or from the death and disintegration of the parasite, whether the parasite remain in the lumen of the bowel or penetrate the tissue and cause direct trauma and parenteral absorption. Any such toxic products although not resorbed would be a menace, so that the harmlessness of these organisms must be proved rather than their pathogenicity defended. The most complex organism is man. With his highly developed nervous system, man is psychically sensitive to the slightest influence, and so sensitive an organism has no "harmless commensal." All parasites, in one way or another, affect him, although it be but slightly. Man's adaptability, marvellously developed, may cover the losses or damages for a time, or even for a lifetime, but in any case there will be individuals who testify to the dangers latent in a parasitism by abnormality of physiology, or by nervous or psychic reaction. Even our inevitable and universal "commensal," B. coli, has had some evidence brought against it as shortening our lives; brief experimental periods have exploded the theory that the intestinal bacteria were necessary to life, and all know that B. coli occasionally develops its potentialities to the point of causing acute illness.

I recall two instances of the absorption of products, definite at least in the effects produced, caused by parasites as far apart as Hymenolepis nana, and Entameba histolytica and trichomonas in a double infection. The patient with the dwarf tapeworm, seen in Panama in 1912, had constant fatigue, with attacks of overpowering somnolence which nearly cost him his position. During one course of four weeks' treatment he passed 72,000 tapeworms (there was of course autoreinfection, period about seven days), and he was never entirely freed from the infection during the three years I was able to follow his case, but was symptomatically relieved and kept in good health by occasional dosage with aspidium. The other patient, Case 17 reported previously,[3] had lethargy with attacks of somnolence during which he would fall asleep while walking, riding horseback, etc. His attacks disappeared with the amebae. Many symptoms in cases of amebiasis can be more plausibly explained and the pathogenesis is more comprehensible if we consider that the metastatic growth of the amebae is less common than the absorption of the products of their growth, resulting in affecting with toxins tissues situated at a distance from the site of the amebic proliferation, such localization in any special tissue being due either to a specific tropism of the tissue cells for the absorbed molecule, or to a greater susceptibility of the tissue due to strain (joints), or structural delicacy (nerve). Barrow[4] says there is a toxemia or systemic poisoning from amebiasis manifested by a disturbance in the skin, joints,

blood-forming organs, endocrine function, metabolism, nervous system, or by psychic balance, a poisoning as definite in its clinical aspects as that of any mineral poison. Toxicity and actual tissue invasion are not always clearly distinguishable with present methods. A case illustrative of the theory of a toxic absorption from amebiasis is that of a dentist, Dr. L., who had attacks of sciatica of increasing severity for over a year, and had had all the laboratory tests, clinical, radiologic and protein tests that are available, all negative. The fecal examination was reserved to the last, and numerous E. histolytica found, containing blood cells. He had never had dysentery, had lived in Kansas before coming to San Diego, and had not had tropical contact. The nerve pain disappeared, lagging a few weeks after treatment of the amebae with stovarsol, but returned in three months, at which time the amebae were found numerous again, no treatment having been given in the interval, purposely. He is now well of his sciatica, and the amebae have not increased to the level at which they can be found in the stool, due to regular short courses of stovarsol. In this case the possibility of an occupational (position) neuritis was ruled out, first, as the pain was right sided only; the strain, however, was probably the determining factor in making this nerve the situs minoris resistentiae for a nerve poison absorbed from the activities of the amebae. It is hardly likely that in cases like this, or like those cases of iritis reported by Mills,[5] occurring "during the course of a nondysenteric amebiasis," that there is an actual invasion of the amebae in the nerve or in the eye, although by the weight of evidence there can be no doubt that the symptoms are due to the amebiasis. Of course amebae have been found in many parts of the body, carried by the blood stream, as can be found in the list of references given by Mills in his paper and by Kofoid and associates in their paper on "Systemic Infections by Entameba Dysenteriae."[6] The finding of amebae in certain cases of arthritis by Kofoid and Swezy[7] has not been confirmed so far, nor has their demonstration of amebae in Hodgkin's disease, due probably to the difficulties and time required for thorough search. With the discovery of better methods of culture the question of the actual presence of the amebae in these and other lesions may be settled; one recalls the difficulty of finding the streptococci in joint lesions before a special technic was developed, and the long time that these arthritic diseases were attributed to a diathesis—for which, one wonders, the present day fashionable synonym may be "toxicity."

In bacterial feeding there is necessarily an excretion of the digestive enzymes in order to liquefy the food outside the bacterial body so that it can be absorbed. These enzymes and the excreta of bacterial catabolism account for nearly all the action of bacteria upon the human body. A pathogenic bacterium producing a large amount of enzyme to digest a resistant food might be said to have an increased virulence. Most of the amebae and flagellates in the human intestine live free in the lumen, and obtain their food, consisting chiefly of bacteria and yeast cells, occasionally cellular detritus or other protozoa, by engulfing it entire into a food vacuole from which it is absorbed. Except for the dejecta of life processes there is no product necessarily excreted by the intestinal protozoa either to obtain their food, which they are able to capture by movement and do not appear to paralyze, or to

predigest it. There is the notable exception of Entameba histolytica which is entirely or almost entirely a tissue feeder,[8] eroding or penetrating the tissues of its host by means of a cytolytic solvent so subtle that the phagocytic cells of the host are not alarmed. Rarely does it ingest blood cells except when present in the dysentery of which it is the cause. But our ignorance of the metabolic processes of the intestinal protozoa amounts to almost totality, due to our inability to grow them in pure culture. Even the histolytic ferment of the ameba of dysentery is known only by its effect, visible enough even in stained tissue sections in the clear liquid zone surrounding the amebae that have invaded the tissues. While we are awaiting more exact information it is best, as Kofoid advises, to record all parasites found in each case, carefully to distinguish the species, to study the history and symptomatology of the patient in the greatest detail, and in general proceed in a manner calculated to increase our knowledge, rather than to pass without notation the infections that seem harmless in present light. We are fortunate to have the enthusiasm and accurate observation of a protozoologist like Kofoid working on these problems in medical territory with resources that promise solutions for many of them.

Omitting the amebae reported from a few isolated cases, six species have been described occurring as parasites in the intestines of man. There is considerable confusion in the nomenclature, so that the older papers are unavailable for the ordinary student on account of the numerous synonyms, ten to twenty for each species, and even today the authorites have not agreed upon the correct names for the different amebae. The ameba of dysentery is Entameba histolytica for Dobell, Endameba histolytica for Hegner and Tagliaferro,[9] Entameba dysenteriae for Brumpt and for Kofoid. Endolimax phagocytoides (B.), Endolimax nana (H. and T., D. and O'C.), is Entameba nana for Kofoid. Iodameba butschlii (D.), Iodameba williamsi (H. and T.), Endolimax williamsi (K.), is Pseudolimax wenyoni for Brumpt. Since the writings of these authors are on the ready reference shelf of laboratories doing fecal diagnosis, it is unfortunate that there is not the unanimity in names for these amebae that there is with Entameba coli. Councilmania lafleuri (Kofoid), not generally recognized yet, is listed by Brumpt[10] among the synonyms of E. coli, which he speaks of as being "totally inoffensive," while giving in a footnote reference to the observations of Riff, of Strasbourg, who found it in cysts of the appendix and of the tubes. In some instances E. coli may have been confused with the pathogenic Councilmania lafleuri, in others it has been sufficiently identified as the cause of an enteritis. Crowell[11] says that the position of E. coli is not certain; some do not concede its lack of pathogenicity, finding cases of dysentery in which only E. coli is found. Brown[12] reports several cases in which E. coli was without doubt pathogenic, and also reports three cases in which Endolimax nana (Entameba nana) seemed responsible for a severe diarrhea. This ameba has hitherto been considered harmless. Dientameba fragilis and Iodameba buetschlii have never been found in suspicious circumstances.

Thus the only regular offender is Entameba histolytica, and until the extensive stool surveys were made during the war, its rôle was seldom ques-

tioned. Then, finding this ameba in large numbers of apparently healthy carriers cast doubt on its pathogenic rôle, and brought about a change of opinion, more among the zoologists than among the clinicians. Hegner[9] says that in spite of E. histolytica living only as a parasite on the tissues, not more than 10 per cent of the people infected show any marked clinical symptoms. The "size races" seemed to offer an explanation, at least in part. Brumpt[10] noted that the "minuta" forms had never been seen in the liver, that they were not blood-feeding, and were the type found in (so-called) healthy carriers and convalescents. Kofoid[13] states that while we have no information as to the meaning of the size races, that when the clinical histories indicate a long standing infection, the small race, with cysts from 4 to 6 mu. in diameter, are found, and are suggestive of a possible modification in size as a result of long contact with the host. "Cases of human infection observed over months, or at intervals of years, reveal a constancy of cyst dimensions indicating that changes do not occur quickly." Kessel[14] found distinct size races in rat ameba which remained constant during passage through culture rats over a long period. Dobell[8] said that the races differing in size differed in no other characteristic, morphologic or physiologic, but he wrote this in 1921. Brumpt, in the 1922 edition of "Parasitologie," describes two cycles in the evolution of the dysenteric ameba; "a normal, nonpathogenic cycle," and the abnormal pathogenic cycle. In the carriers the ameba is always of the small size race, multiplies by scission, and forms the cysts with four nuclei. The abnormal cycle "is brought on by the influence of intercurrent disease, parasitic associations, or following modifications of the intestinal status" or changes in the general health of the host, which cause the ameba to develop tropisms unfavorable to the host. This transformation, exceptional in man (2 to 3 per cent), is the rule in the cat when it is infected by the ingestion of ripe cysts. The small races will cause dysentery in cats, he states, yet no one has explained how the minuta forms become hematophagic and pathogenic. "The hematophagic amebae multiply rapidly by scission and are rapidly eliminated with the quantities of mucus coming from the intestinal irritation which they provoke." The question of size races is much confused. The change in size on feeding cats with cysts of races with small cysts, should be confirmed by the careful measurement of ripe cysts later obtained from the cat, to determine whether the small precystic ameba had not merely grown into the larger, hematophagic, rapidly multiplying form, and did not again produce small cysts when conditions again favored encystment. The same change in size of motile forms can be brought about in culture amebae by a meal of red blood cells. Reports from various workers that they have "seen the small race change to the large during a relapse of dysentery," and vice versa, have not been based on cyst measurements. Again it is not infrequent that the two size races exist simultaneously, a larger ameba with large sized cysts ingesting blood cells and the patient suffering from dysentery, while a small race with small cysts coexists, of uncertain rôle. Such was the case of a patient recently referred by Dr. Alberty for fecal examination. The patient had never lived away from the northern Middle West, and had had dysentery for about a year. In this case we do not know but that this man had long been

a carrier of the small race of E. histolytica, and had a secondary infection with the larger race that produced the dysentery the past year.

There seems to be a distinct difference in the pathogenicity of the size races. The small races are widely diffused throughout all races of mankind, and family infections are common. Kofoid[13] says that patients reporting no history of dysentery form by far the greater proportion of their records, but adds that "such testimony could hardly be expected to represent accurately the period of infancy, within which amebiasis may well have been acquired, and may have been accompanied by an initial attack of dysentery followed by the chronic carrier stage, whose most evident intestinal symptom is constipation." In many examinations of the stools of infants and children with diarrhea and dysentery in California, I have never found amebae, although about 15 per cent of adults referred for examination because of intestinal symptoms are carriers of E. histolytica. Certainly the small races do not ingest blood cells and in the nondysenteric amebic diseases are the type usually found. In the case of "arthritis deformans of Ely's second type, and of Hodgkin's disease, as well as many cases of chronic, low-grade ill health," Kofoid[6] finds the size of the cysts generally less than 10 microns, often 7 to 9, sometimes 6 to 8, rarely from 3 to 5 microns. Such small amebae could pass through any capillary.

Until the culture of the ameba is simpler than it is at present, the problem cannot be solved whether the large forms are activated hematotropic ameba of the same race as the small amebae, or really a different infection. "In any case, this question of races presents a considerable biologic interest for the problem of immunity," says Brumpt;[10] "indeed, if there really are different races, it might be asserted that the infected individual is in a state of anergy, since, in spite of the new infections by cysts from races differing in size, he shows only the race that infected him originally. On the contrary, if the dwarfism or giantism of the cysts is due to the surrounding medium, it is impossible for us to know whether superinfection is possible or not, and whether there is a relative immunity in the course of intestinal amebiasis." As above stated, we do find individuals with coexistent different size race infections, but have no means of telling whether the one size race has not developed from the other. The possibility of immunity in the rat, Kessel thinks, is suggested by his experimental facts. Dobell and O'Connor[8] say that all the evidence goes to show that whether the infected individual suffers or not from his infection depends rather on his own susceptibility than on the virulence of the parasite. Haughwout[2] says that it is hard to escape the conclusion that the host on occasion may transform an apparently harmless parasite into one that is pathogenic, apart from lowered vitality and resistance.

There is today little doubt that E. histolytica is more or less pathogenic to any individual who harbors it. Dobell says E. histolytica is a true tissue parasite, facultatively pathogenic. "There can be no doubt that the carrier of E. histolytica, though he display no symptoms, always has a more or less eroded or ulcerated gut." "Amebic infections are very persistent, probably life-long, unless eradicated by specific treatment. Consequently all who once become infected with this parasite are likely to suffer from amebic diseases at

some subsequent time. For the average case the risk is probably small." There was lacking until quite recently the thorough search for symptoms in the past or present of such carriers and the proof that, when the number of amebae has risen to the level at which they may be found in a fecal examination, they are numerous enough to cause symptoms. This work has largely been done by Kofoid and his followers.

We have long known that the ameba living in the mucosa of the colon invaded the vessels and found its way to the liver, and occasionally to the lungs, usually by extension, and rarely was carried in the blood to the brain. Later, amebae were found in the urinary tract, testis, fallopian tubes, and spleen, and finally in the bone marrow in certain cases of arthritis, and in the lymph glands in Hodgkin's disease by Kofoid, Boyers and Swezy,[6] who "connect this ameba with a widely prevalent mild type of invalidism in middle age and thereafter."

Boyers, Kofoid and Swezy[1] state their present concept of chronic amebiasis with E. histolytica (vel E. dysenteriae) as a definite clinical entity, recognizable as such. They find a marked fatiguability, commonly associated with constipation or a constipation broken by evanescent diarrheas, abdominal soreness, digestive disturbances and neurosis of vague nature. "The normal man knows hunger and the desire for evacuation, otherwise his bowel does not obtrude itself on his consciousness. In amebiasis he is not comfortable in the abdominal region, he is "bowel conscious"; as a rule he has been previously diagnosed as having chronic inflammation of various organs, and sometimes, unfortunately for the patient and for the reputation of the operator, there is a history of operations without relief; in Barrow's cases,[4] 28 per cent had had gastrointestinal operations. "Human amebiasis is a definite disease entity, protean in character as is syphilis, undramatic in behavior, subtle in onset, and definitely nonbacterial in type. The protozoa are minute animal forms often tolerantly regarded by human tissue for a long time." Chronic amebiasis is nearly always an infection by the races with small cysts, and there is almost never a history of a dysenteric attack, rarely that of residence in the tropics or contact with people who might have brought back as convalescent carriers an amebic race that is known to have caused dysentery.

With matters standing thus, an added interest and a fresh turn given the discussion by an attack on the pathogenicity of the small races of E. histolytica by the author of the laboratory "bible" of parasitology, Professor Brumpt, who left the question doubtful in the last (1922) edition, while citing his own belief at the time as for the mutation of size races. In a preliminary note,[15] he calls the attention of physicians to an "ameba generally confused with the ameba of dysentery, Entameba dispar."

The small race of E. histolytica, based on its wide diffusion throughout mankind and its being nondysenterigenic, set it aside as a new species. E. dispar, which Brumpt describes as resembling E. histolytica morphologically, in appearance, color, clear pseudopodia and faintly discernible nucleus, differing in the negative signs of the absence of the large motile forms, even after purgation, the absence of red blood cells in the food vacuoles, and slightly less motility at room temperature; all points previously noted by

Brumpt and others as characterizing the small race of ameba. The cyst is like that of E. histolytica in number and appearance of nuclei. E. dispar is ranked as a new species (a) on the basis of its food, bacteria and yeasts,* (b) its slight pathogenicity for the cat, in which it produces but a fugaceaous lesion, never dysentery, and on a (c) second biologic ground of being widely distributed and not causing dysentery. He quotes statistics that show that in England 5 per cent of the population are carriers of the ameba with four nucleate cysts, in France 4 per cent, in the United States 5 per cent, in Venezuela 30 per cent, in Buenos Aires 24 per cent. "Statistic studies show that the amebae with quadrinucleate cysts are as common in the stools of people inhabiting countries where amebic dysentery is rare as where it is frequent. Why is the ameba dysenterigenic for man once in four times in the Philippine Islands, 1 in 20 in Macedonia, Indo-China, the Senegal, and Morocco, while in England with approximately 2,000,000 carriers but one or two autochthonous cases arise during the year?"

Sohei Shimura, a Japanese writer, in 1918 noted the presence of an ameba "not pathogenic" for man in six individuals, five "with a normal intestinal tract, one with a nondysenteric chronic catarrh." With this ameba Shimura was able to give only a transitory lesion to 3 of 23 kittens inoculated, while he obtained positive results in 91 of 100 inoculated with amebae from dysenteric stools, and 50 in 100 making the kittens ingest cysts from dysentery. Similar experiments by Brumpt himself have changed his opinion, expressed in the 1922 edition of the "Parasitologie" that the small forms had a low infectious power; to the theory that the difference is that there is a nonpathogenic species. Brumpt and Drbohlav in the former's laboratory, using the technic of Boeck and Drbohlav† were able to infect kittens from healthy carriers. In thirty kittens Brumpt found no ulcerations, only a few congested areas, although the amebae were extremely numerous, occasionally they contained red cells, but there was no true dysentery.

The surprising epidemiologic facts are much easier to understand, Brumpt thinks, if it is admitted that the widespread ameba is not dysenterigenic for man, "perhaps not even pathogenic," and should be considered a species different from the dysenteric ameba, the frequency of the latter having no relation to that of the quadrinucleate cysts. The morphology similar to that of the ameba of dysentery need not hinder our making the distinction, "for Prof. Dobell is incapable of distinguishing E. ranarum from E. histolytica, either in the vegetative form or in cysts, but separates it by biologic character." Indeed, the statistics for amebiasis are being found to hold true for peoples like the Alaska Indians who have had no possible contact with the tropics, and the difference between the number of carriers and the morbidity from dysentery is so great one might well be surprised. There is a difference of opinion as to the food of the small ameba. Only the large amebae ingest blood cells. The mode of nutrition, says Dobell, "is peculiar in this species, being mainly by absorption, and bacteria are probably never ingested by nor-

*In a case with diarrhea I studied with the small race of ameba 90 per cent contained bacteria, none blood cells.

†Rectal inoculation under ether anesthesia, and sealing the kitten's anus for twenty-four hours. Using this technic with amebae from two carriers with symptoms but who never had dysentery, I was unable to obtain even implantation in young kittens.

mal individuals." Hegner and Tagliaferro seem to conclude that the amebae in man other than E. histolytica, having as food bacteria, yeasts, and never blood cells, are evidently harmless commensals. Food is not necessarily a criterion of pathogenicity, an analogy no worse than a deduction from the visible food content of a protozoan, is the pathogenicity of the streptococci, which are not all hemolytic by any means. The epidemiology is still more perplexing when one notes with Brumpt that the ameba from carriers does not cause dysentery even after violent purgation, a fact in line with the comparative difficulty of implantation in young cats.

In his lectures at the School of Medicine in Paris, Brumpt groups E. histolytica into three size races. By noting the cysts on a diagram with the size as abscissa, the cysts will be found to describe a curve, with common races the height of whose curve (largest number of cysts) will be about 8 mu, another at 15 mu, with a less frequent race averaging 12 mu. "It is the small race with cysts about 8 mu that is E. dispar," although one not familiar with the rules of zoologic nomenclature would wonder why "E. minuta," described by Elmassian in 1909, would not take preference.

Certainly all will agree with Brumpt that amebic dysentery is only an episode in amebiasis. There is a large race of E. histolytica which is found in dysentery, common in the tropics, excessively rare as a disease originating in the temperate zone; and a small race that is common among mankind everywhere, and is accompanied by a symptom complex resembling a toxicosis, occasionally with the finding of amebae in various organs of the body. Dobell[5] thinks there may be some kind of natural resistance, that the pathogenicity of the ameba depends rather on the patient's susceptibility than upon the virulence of the parasite. This does not wholly explain the facts. The enormous number of carriers in northern countries with neither dysentery nor history of it, compared with the frequency of dysentery in the tropics, makes too wide a discrepancy to account for simply by tropical neurasthenia. Nor does the ameba seem to gain virulence by animal passage, as with so many pathogenic bacteria, which would explain the incidence of acute dysentery in the tropics, where climate preserves the vitality of the cysts for a longer time, and bad hygienic conditions favor frequent passage, besides the common state of undernutrition in the favored lands under the sun, where ease makes for sloth. We cannot, in view of the facts abduced by numerous investigators, agree with Brumpt that the small race of the "dysenteric" ameba is not pathogenic, but we must acknowledge that it is quite different in its pathogenicity from the large, and agree that it is of identical appearance. It is possible that "nonpathogenicity" of the small races as seen by Brumpt, Dobell and other European observers is because their patients are more stoical, less apt to note or complain of the discomforts of life, more likely to accept them as the inevitable accompaniments of advancing age, than the American with whom luxuries have become a necessity, and who, somewhat softened by gynarchy, is attempting, and in this case with some success, to feel "as young as he ever was." However this may be, it is certain that the small race while rarely causing dysentery, and of unknown relationship to the larger race, is in nearly every carrier, the effective cause of some degree of disease.

As Mills says of his eye cases, the sole addition of antiamebic treatment results in the cure of most of the otherwise obscure inflammatory disorders in patients with heavy intestinal infections. The argument that would carry most weight, that of the healthy carrier, is the one being contradicted by workers in many places, and particularly by Kofoid and his followers. The latter state that there are no "healthy carriers." And this conviction is spreading. Simon[16] discussing the comparative frequency of infection with E. histolytica, abduces the fact that surgeons have long recognized that liver abscess may exist, without intestinal symptoms, and states that "carriers of so-called symptomless E. dysenteriae generally show some deviation from the normal." Brown[17] thinks amebae are a factor in obscure abdominal complaints associated with neurasthenic symptoms. He says the patients in his group 3, carriers with no history of dysentery, are likely to have dysentery following a major operation or a debilitating illness. The carriers of the ameba that occasionally cause a disease so dangerous are truly widely disseminated among the people of all countries, but is there not also a widespread morbidity, not often troublesome enough for the formal attention of a physician or other healer, but sufficient to support, along with many religions of healing, an immense and wealthy patent medicine business which consists nearly entirely in the dispensing of digestants and laxatives. It would be too much to presume that the carrier population coincided with the population with halitosis and constipation, yet the commonest effect of chronic amebiasis is constipation.

These amebae, that are so common in all races of men, cannot have been spread from carriers from the tropics. We must presume that this generally diffused infestation has existed in the races of man now inhabiting the temperate zone (to whom we are confining our attention for the moment) for so long a time that the organism and its host have ceased active warfare, the harm to the host being reduced to that of the nonfatal disabling affections, dysentery occurring rarely in subjects peculiarly susceptible or under unusual conditions. In the "carriers" we find (toxic?) chronic neuritides, arthritides, irregularities of intestinal functioning, that are the more common among the infected the more closely we question and examine them. The amebae (not only E. histolytica) have been parasitic in the digestive tract since the now northern races left warmer climates at an early period in the history of man. With so long an association it is not surprising that host and parasite live together in comparative harmony. But there are facts that suggest that the relationship is immensely older. In the monkey there are two types of parasitic amebae, exactly resembling E. coli and E. histolytica, even to the nonpathogenicity in the case of the ameba resembling E. coli, and to the causation of dysentery and liver abscesses by the ameba resembling E. histolytica. These amebae, Dobell thinks are identical with the human species, and if so, furnish a missing link of parasitic nature of great interest. With so ancient a companionship, it is inevitable that there should be a tendency toward fixation of a biologic character favorable both to the ameba and to man, which, while not necessarily presenting the differentiation of a definite species, yet might be legitimately regarded as a species in the process of formation. With a change in function there is usually an accompanying change in

morphology, slight in the higher organisms, more marked in the simpler. The change in size may be a mark of the change in character.

The differentiation of Councilmania by Kofoid and Swezy,[18] has cleared a source of confusion, in the vegetative stage with E. histolytica, in cysts with E. coli. Similarly further differentiations may be expected in this new field of work. New staining methods, but chiefly, the cultivation of amebae, will enable clinicians to make more exact diagnoses. E. dispar, even if not a valid species, shows a partial fixation of character similar to that of the streptococcus races with joint, gall bladder, throat or other tissue localization. It is too early to draw any conclusions. Although enough facts may not have been adduced to prove E. dispar has a right to exist as a separate species, at least there are enough to show a tendency, in a protozoan parasitic in the primate stock since its origin, toward the formation of a variety with fixed habits. But raising the question of the specificity of the ameba present in carriers, and Brumpt leaves it a question, will direct study by fixing attention upon definite points of attack in the ameba problem. More careful study of fecal protozoa is needed, far more than the customary separation of E. coli and E. histolytica. All species must be noted as found. We are still in the statistic stage with the protozoa, the stage of uncertainties, and of arithmetic errors, but a stage of hope. E. dispar, not mutable into a dysenterigenic species, or very exceptionally so, may exist, as species etiologic of much chronic illness. The mutability of the size races has yet to be proved. Between cultivation and animal experiments this may be determined, but human experiments may be necessary. A problem named is already half solved.

SUMMARY

1. There is evidence that the intestinal protozoa cannot be beneficial to man, only negative evidence that they are ever innocuous, and positive evidence that they can be dangerous; theoretically any of them might become pathogenic. The danger of an infection may be shown either by time of observation in the individual, or by the percentage of disease in a number of infections.

2. Entameba histolytica presents two types: a race with large cysts usually associated with amebic dysentery of tropical origin, and a race with small cysts that is common everywhere, rarely associated with dysentery, but apparently the cause of low grade illnesses in midlife. The most complete proof of the etiologic relationship of the ameba to this pathogenicity has been given by Kofoid and his followers.

3. Brumpt thinks the small race differentiated enough to be considered a separate species, E. dispar.

4. The question of mutability or immutability of the size races of E. histolytica awaits perfection of culture methods and media, its growth in pure culture free from bacteria, preferably with living tissue cells to approximate natural conditions. Study could then be made of the histolytic enzyme or other products. Surveys of different age-groups would show at what time of life amebic infection begins.

5. The effect of tropical climate on man and its possible reaction upon his parasites, suggested by Haughwout, is important. Roddis and Cooper[19] have shown a definite harm from tropical climate, lowered basal metabolism, blood pressure, suggesting the possibility of other variations.

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CORYNEBACTERIUM ULCERANS: A PATHOGENIC MICROORGANISM RESEMBLING C. DIPHTHERIAE*

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THE literature contains many references to diphtheria-like bacilli which have been isolated from various sources. Such microorganisms have been found to vary in morphology from coccoid to bacillary forms and to differ markedly in pathogenicity. The microorganism to be described here exhibits definite morphologic and cultural characteristics, some of which have been ascribed to certain microorganisms previously reported. The name *Corynebacterium ulcerans* is proposed for this species.

Klein[1] described a microorganism that was isolated from an indurated udder of a cow. Abscesses were produced when guinea pigs were inoculated with the bacilli. The cultural characteristics, as well as the slowness with which the lesions developed in animals that were inoculated, indicate that the culture belonged to a different species from the one reported here.

The *B. scarlatinae* reported by Mallory and Medlar[2] varied in morphology from coccoid to long bacillary forms but, during the primary cultivation, grew only under anaerobic conditions. It produced no soluble toxin.

The virulent pseudodiphtheria bacilli described by Ruediger[3] and Hamilton,[4] and the *B. enzymicus* reported by Mellon,[5] all produced a septicemia in inoculated animals.

The virulent poison-producing diphtheroids described by Parker[6] resemble very closely the cultures that we have been studying. The similarity will be discussed later.

The first culture of *Corynebacterium ulcerans* was isolated in January, 1920, from a patient who had had diphtheria the preceding October and had recovered clinically in December of that year. In subsequent cultures from this patient received in February and April, a similar bacillus was found. Since that time, thirty-six such strains from thirty-one different patients have been isolated during the course of the routine tests; in some cases, very little of the clinical history could be obtained.

MICROSCOPIC STUDY

The rapidity with which the microorganism changes its morphology was one of the first characteristic features to be noted. In young cultures (four to eight hours), the organisms closely resemble *C. diphtheriae* in morphology, some being uniformly stained, others having polar bodies. In older cultures (twenty-four to forty-eight hours), most of the forms are coccoid. In mixed

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cultures, however, more of the bacillary forms may be found after the longer incubation period. In order to insure the purity of the cultures, fifteen of the strains were isolated from single cells by Ørskov's[7] method.

A study of these organisms shows that they are nonmotile and do not form capsules or spores. They stain readily, retain Gram's stain, and are not acid-fast. Polar bodies can be demonstrated in young cultures by Neisser's, Albert's, and Loeffler's alkaline methylene-blue, stains.

CULTURAL STUDY

The microorganisms are facultative anaerobes that develop readily in the usual laboratory media. In general, the colonies are larger and more opaque than those of *C. diphtheriae* and, on dextrose serum agar, they vary in diameter from 0.25 mm. to 1.0 mm. They have slightly irregular edges. On blood agar, they produce very slight hemolysis. A pellicle is formed on ascitic fluid and on dextrose beef infusion broth. Dextrose, maltose, and dextrin are fermented with the formation of acid and a coagulum, in serum-water medium. Mannite, saccharose, and lactose are not fermented. Litmus milk inoculated with cultures shows little evidence of change. Indol is not formed after three days' incubation (Ehrlich's and Salkowsky's methods), and nitrates are not reduced. Gelatin is liquefied in from six to seven days. The microorganisms are killed by heating at 56° C. for ten minutes.

VIRULENCE AND PATHOGENICITY

When 0.2 c.c. of a suspension of a pure culture having an opacity equal to barium sulphate standard No. 1½ are injected intracutaneously into a normal guinea pig and also into one which has received previously five hundred units of diphtheria antitoxin, a very definite area of induration, congestion, and necrosis appears in from eighteen to twenty-four hours on both animals. When the entire twenty-four-hour growth on a Loeffler's blood serum slant is injected subcutaneously into guinea pigs, one of which is normal and one of which has received five hundred units of diphtheria antitoxin, a very marked reaction showing extensive necrosis, followed by the formation of an open ulcer, usually appears at the site of injection on both animals. Occasionally, one or both of the animals in such series dies. The animals that have not received antitoxin die more frequently than those that have.

The most characteristic lesions noted at autopsy are the fibrinous exudate, very marked congestion and necrosis at the site of injection. There is slight or no congestion of the adrenals. Varying degrees of congestion are found in the stomach and intestines. Occasionally, the intestine and cecum may be almost covered by petechial hemorrhages. No abnormalities occur in the liver and kidneys, and excess fluid is seldom found in the pleural cavities; but a large amount of pleural fluid is found in animals inoculated with some of the cultures. The organisms are readily recovered from the site of inoculation but never from the heart's blood.

When examined histologically, there is ulceration of the skin at the site of inoculation and marked serous and polymorphonuclear infiltration of the

subcutaneous and muscular tissue; there is practically no fibrin present in the exudate. Sparse Gram-positive organisms having the morphology of both cocci and bacilli are scattered through the inflammatory area. Slight or moderate congestion with occasional minute hemorrhages occur in the medulla of the adrenals. In the other organs, moderate degrees of acute congestion are found.

The chief differences, therefore, from the lesions induced by *C. diphtheriae* are seen in the uniform occurrence of ulceration at the site of subcutaneous inoculation and the absence of diffuse hemorrhagic infiltration of the adrenals.

Guinea pigs inoculated intraperitoneally with a forty-eight-hour broth culture of the *Corynebacterium ulcerans* die almost consistently from twenty-four to forty-eight hours later. At autopsy, a purulent exudate is usually found in the peritoneal cavity, as well as multiple small abscesses scattered through the omentum and liver.

The local reactions on rabbits resulting from both intracutaneous and subcutaneous injections of the microorganisms are always more severe than on the guinea pigs. The lesions are more extensive, and the more intense areas of induration, congestion, and especially necrosis are followed by the formation of an ulcer. White rats are less susceptible than guinea pigs.

TOXIN PRODUCTION

Broth cultures of this microorganism were filtered after seven days' incubation at 37° C. The veal infusion broth for these cultures is the same as that used in the preparation of diphtheria toxin. The potency of different lots of this filtrate varies, and there seems also to be a definite variation in the susceptibility of the guinea pigs to the toxin, since, at times, 0.1 c.c. injected intracutaneously may induce only a small area of induration and congestion, while again an extensive local reaction with an area of necrosis may result.

The filtrate is much more toxic for rabbits than for guinea pigs. Whereas 0.1 to 0.2 c.c. usually is required to induce a reaction about 1 cm. in diameter in guinea pigs, 0.01 c.c. injected intracutaneously into rabbits results in a raised area of congestion and induration varying from about 2 to 2½ cm. in diameter.

An injection of 2 to 3 c.c. of the toxin, subcutaneously, into guinea pigs may result only in the formation of an area of induration and congestion 3 to 4 cm. in diameter, while, after an injection of 5 c.c., a definite area of necrosis appears in the center of such a lesion. Occasionally, animals receiving the larger inoculum may die. The autopsy findings are similar to those of animals inoculated with living culture. The lesions induced by subcutaneous injections in rabbits are more severe than those in guinea pigs, 0.5 c.c. resulting in a marked local reaction.

Guinea pigs injected with even as much as 5 c.c. of the toxin usually survive, while a dose of 0.5 c.c., either intraperitoneally or intravenously, usually proves fatal for rabbits.

Mice injected with 0.5 c.c. subcutaneously or intraperitoneally survive, and no lesions are noted.

The toxin is destroyed by heating at 56° C. for ten minutes.

IMMUNE SERUM

Since reactions were obtained which indicated that the organisms formed a soluble toxin, an attempt was made to produce an antitoxin by the immunization of a horse. This animal received inoculations of gradually increasing amounts of the toxin at three-day intervals for eighteen months, except when trial bleedings were taken. An inoculum of 800 c.c. was used during the last eleven months. At the end of eighteen months, a serum was obtained $\frac{1}{150.000}$ c.c. of which neutralized 0.01 c.c. of the toxin when injected intracutaneously into rabbits. Approximately 0.5 of a unit of diphtheria antitoxin neutralized this amount of the toxin, while 0.1 c.c. of normal horse serum had no neutralizing effect. Guinea pigs were protected against the local reaction induced by a subcutaneous injection of 5 c.c. of the toxin, when $\frac{1}{2.000}$ c.c. of the antiserum was injected with it. Likewise, 300 units of diphtheria antitoxin protected against the reaction, while 1 c.c. of normal horse serum gave no protection.

Before inoculation, the serum of the horse contained less than $\frac{1}{500}$ unit of diphtheria antitoxin per c.c. After immunization for thirteen months, however, approximately 30 units per c.c. were found to be present. Although immunization was continued, the diphtheria antitoxic content of the serum decreased from this amount until there were approximately 5 units at the time the last serum was obtained from the animal.*

Although satisfactory protection was obtained against the specific toxin by the antiserum, the results were not so satisfactory when tested against cultures of the organism. When these cultures were tested intracutaneously in guinea pigs which had previously received 1 c.c. of the antiserum, the local reactions were only slightly diminished from those obtained on normal animals or on those which had received normal horse serum or 500 units of diphtheria antitoxin. When the twenty-four-hour growth of the organisms from a Loeffler's blood serum slant was tested subcutaneously in an animal which had previously received 1 c.c. of the antiserum, the local lesion was reduced from an extensive area of induration, congestion, and necrosis of from 4 to 5 cm. in diameter to one of induration and congestion about 2 cm. in diameter. The degree of protection was not the same with all the strains studied, for with some a small area of necrosis developed.

The antiserum was tested against cultures of virulent diphtheria bacilli as well as against diphtheria toxin, and some protection was obtained since this serum contained as much as 30 units of diphtheria antitoxin per c.c.

HUMAN TESTS

A few intracutaneous tests of the broth filtrate have been made on adults. Four people who had failed to react to the Schick test and were inoculated

*In the few instances in which the serums of horses which had been immunized with pneumococcus, streptococcus, and tetanus toxins were tested for their diphtheria antitoxic content, there was little or no increase in the titer.

intracutaneously with 0.1 c.c. of a 1:10 dilution of the toxic filtrate, developed definite areas of congestion, varying in extent. One such reaction, which measured approximately 18 mm. in diameter after twenty-four hours, had faded in the next twenty-four hours so that only a faint area of pigmentation remained. The other reactions were more extensive—one which measured 5 × 4.5 cm. after twenty-four hours had extended to 6.5 cm. in the long diameter after ninety-six hours, and marked pruritus was present.

Five people who had reacted definitely to the Schick test were given half the amount of filtrate. These, too, showed areas of congestion but not more extensive than those already described. Three of these faded, leaving a pigmented area after seventy-two hours; one remained more definite, while the others showed slight erythema for several days. All of these individuals failed to react to the heated filtrate.

The neutralizing properties of antitoxin for the toxin were tested in a few instances. The undiluted horse serum protected against an equal amount of a 1:10 dilution of the toxic filtrate; and a 1:20 dilution of the horse serum seemed to give almost complete protection against an equal amount of a 1:20 and a 1:10 dilution of the filtrate.

CLINICAL HISTORY OF CASES

In connection with this part of the work, it may be of interest to review the clinical histories of the patients from whom these thirty-one strains were obtained. Five of the cultures were isolated from cases diagnosed as diphtheria. These were submitted from various periods in the course of the disease, from thirteen weeks after onset in one instance to forty-eight hours in another. One culture was from a child about whose clinical condition no data were obtainable. Throat cultures from his sister contained virulent diphtheria bacilli. Six cultures were from patients with symptoms of a "cold" or tonsillitis, none of whom were ill over a few days. Eighteen cultures were from persons who were not ill, the specimens being collected in connection with surveys for carriers of *C. diphtheriae*. The tonsils in one case were considered enlarged, and, in another, it was stated that they were hypertrophied and diseased. One culture was sent for diagnosis, but, as in the other instances, no information concerning the clinical condition of the case could be obtained.

It may also be of interest to know that, about ten days after the preparation of one lot of toxin, one of the workers who had handled the cultures had a slight sore throat, and a culture of *Corynebacterium ulcerans* was isolated from it. The organisms persisted for about three weeks, although the throat was not sore after the first day.

DISCUSSION

A study of these cultures reveals a group of microorganisms resembling, in only a few respects, other diphtheria-like bacilli reported in the literature, except those of the "poison-producing diphtheroids" of Parker with which they appear to be closely allied. They differ somewhat from the latter in that the organism which he described did not have polar bodies and did not grow satisfactorily on ordinary media, luxuriant growth being obtained only when blood was present. The organism isolated in this laboratory grows well on plain agar, and polar bodies can be readily demonstrated. Parker does not record the reaction in gelatin. In general, the pathogenicity and toxin production of the cultures described by him correspond very closely to those studied here. He did not mention, however, that diphtheria antitoxin was present in the antitoxic serum. He stated that, whereas the necrosis induced

by intracutaneous inoculations of the microorganisms is conspicuous after twenty-four hours, the reactions subside more rapidly than those induced by *C. diphtheriae*, while in our experience the lesions may persist for a week or more.

SUMMARY

The microorganisms here described differ from true diphtheria bacilli in the following respects:

1. Rapid change in morphology from bacillary to coccoid forms usually in twenty-four hours.

2. Liquefaction of gelatin.

3. Nonreduction of nitrates.

4. Reaction from intracutaneous injection of microorganisms on both normal guinea pigs and on those immunized with diphtheria antitoxin.

5. Formation of extensive ulcers on animals inoculated subcutaneously with a living culture.

6. Production of a soluble toxin for which a neutralizing antitoxin was prepared. The serum of the horse thus immunized showed a definite, but limited, increase in its content of diphtheria antitoxin.

Although the *Corynebacterium ulcerans* differs from the true diphtheria bacillus in these respects, it is doubtless a closely related species. From the evidence of pathogenicity that the cultures manifest, it seems probable that it may be of etiologic significance in some of the inflammatory or ulcerative lesions, especially those of the nose and throat.

For much of the technical work connected with this problem we are indebted to Miss H. H. Owen and Miss F. A. Fitzgerald. We also appreciate the assistance of Dr. C. A. Griffin in directing the tests for determining the diphtheria antitoxic content of the antiserum.

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OBSERVATIONS ON THE RELATIONSHIP OF THE WASSERMANN REACTION, CELLS AND GLOBULIN CONTENT, AND THE COLLOIDAL GOLD PRECIPITATION REACTION OF SPINAL FLUIDS IN SYPHILITICS*

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A COMPLETE examination of the cerebrospinal fluid in lueties consists usually of the following laboratory tests:

Qualitative and Quantitative Globulin Increase Test
The Wassermann Reaction
Cell Count
Quantitative Estimation of Albumin
Colloidal Gold Precipitation Test.

The variations of results of these individual tests in different clinical pictures of neurosyphilis have already been extensively reported by Nonne,[1] Eskuchen,[2] Pappenheim,[3] Ravaut,[4] Sicard,[5] Jeanselme, Vernes and Block,[6] With,[7] Schou,[8] and numerous other workers. It does not appear from a review of literature that the results of these individual tests have been comprehensively studied in comparison with the specific Wassermann reaction in neurosyphilities, either before, during, or after specific treatment was begun. An inquiry into the relations between these laboratory tests in luetics presented the following questions: (a) To what extent is agreement or disagreement obtainable in the usually performed examinations of the cerebrospinal fluid? (b) Does any parallelism exist between the Wassermann reaction and other tests and what conclusions may reasonably be made as to the value of the Wassermann reaction in cerebrospinal fluids?

Material: The material employed for comparison of the Wassermann reaction with other tests consisted of 200 cerebrospinal fluids, obtained partly from patients suffering with neurosyphilitic lesions, and partly from patients presenting the clinical picture of latent syphilis. No discrimination was exercised in the selection of the spinal fluids employed in these studies. Every specimen of spinal fluid sent to our laboratory during a definite period from the Clinic of Nervous Diseases at the Riks Hospital, and Department IV of the City Hospitals at Ullevaal, was included in these studies. The material was thoroughly examined by the five ordinary tests for cerebrospinal fluid and their reactions were collated in order to find any possible correlation between the results of such tests. Care was taken to record the results both qualitatively and quantitatively.

Our inquiry appeared to be of considerable interest to the clinician as well as the laboratory worker. It will always be useful to have on record any

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definite correlation of serologic reactions in syphilis. Occasionally the simpler reactions may forecast the results of more extensive and complicated tests. The question often arises how large pleocytosis may a spinal fluid present before one may reasonably expect to find a positive Wassermann reaction, or in what concentration of globulin may one expect the Wassermann reaction to become positive? It is likewise of considerable interest to ascertain whether any relationship exists between a positive colloidal gold precipitation test and a positive Wassermann reaction; and again, the question may be asked, which of these two reactions will first disappear and on which test ought the chief emphasis be placed when a spinal fluid should be considered normal?

Technic: 1. *The Wassermann reaction.*—The amounts of spinal fluid employed for the Wassermann reaction are always 1.0 c.c., 0.5 c.c., 0.2 c.c., and occasionally 0.1 c.c. To avoid unspecific inhibition in the spinal fluid, our routine has been to study spinal fluids without the addition of extracts. In a long series of investigations, we never encountered specific inhibition and hence feel justified in stating that a fresh sample of spinal fluid, not contaminated by long standing, never absorbs the complement in the Wassermann reaction by itself, without the presence of extract.*

All the spinal fluids were examined without inactivation by heat, and in this respect unlike our routine with the blood sera. The extracts employed were prepared according to Kolmer,[9] partly from human and partly from ox hearts. After the alcoholic extraction of the muscle was completed, 0.2 per cent of cholesterin was added to one portion of the extract.

The performance of the Wassermann reaction was as follows: To the amounts of spinal fluid, in duplicate order, were added the previously titrated dose of normal heart extract and cholesterinized heart extract, together with the similarly titrated dose of complement.

Different authors have claimed that the use of cholesterinized extract in spinal fluids was confusing on account of its tendency to self-inhibition. Using only 0.2 per cent of cholesterin, according to Kolmer,[9] and not 0.4 per cent, according to the German workers, we never found self-inhibition in the cholesterinized extracts strong enough to involve any risk. Hence, we disagree with those authors who are hostile to the use of cholesterinized extract in performing the Wassermann reaction in spinal fluids. On the contrary, this extract is as valuable in the Wassermann reaction in spinal fluids as it is in the Wassermann reaction of blood serum. We find that the two extracts give uniform results, although the cholesterinized extract gives a stronger absorption and as such gives a more protracted positive Wassermann reaction in the course of treatment than does the plain heart extract. It appears, therefore, that the result of the Wassermann reaction performed with the cholesterinized extract must be finally relied upon for the decision whether a spinal fluid gives a positive or a negative Wassermann reaction under treatment.

*After this paper was written, we examined a spinal fluid obtained by puncture of the lateral ventricle in a case of cerebral tumor. This fluid gave complete complement absorption in doses of 1.0 c.c., 0.5 c.c., and 0.2 c.c. without nonspecific inhibition and with negative Wassermann reaction in the blood serum. A week later we received a sample of the patient's spinal fluid for examination. The Wassermann reaction was negative. Lues was not present and the reaction of the ventricular fluid was not specific, although it absorbed complement only in the presence of the Wassermann reaction extract. This case is the only instance on our records of nonspecific inhibition in the cerebrospinal fluid.

The incubation of our tests was for half an hour on the water-bath constant at 37° C., and reading was carried out immediately after hemolysis of the blood cells, i.e., when all the controls were thoroughly dissolved.

The Wassermann reaction was performed by this method in a number of spinal fluids from patients presenting the clinical picture of various nervous diseases of luetic and nonluetic origin. Agreement of the Wassermann reaction with the clinical diagnosis, makes it necessary to briefly summarize the material examined. This material contained beside the before mentioned 200 spinal fluids, 251 spinal fluids examined during the same period and sent from different sources to the Army Bacteriological Laboratory of Norway for the Wassermann reaction.

The 451 spinal fluids fall into the following groups: (a) 84 fluids from patients with nervous diseases probably of nonluetic origin. Among these, 83 gave negative Wassermann reactions, and 1 gave a positive Wassermann reaction. The only spinal fluid with a positive reaction came from a patient suffering with Ménière's disease (aural vertigo), the patient admitting a luetic infection twenty years previously. (b) 94 fluids were sent us from patients without clinical diagnoses. Among these, 88 gave negative and 6 positive reactions. Nothing further was known clinically about the 6 positive cases. (c) 116 fluids came from patients with manifest neurosyphilis. 98 of these gave positive Wassermann reactions and 18 gave negative reactions. Several of the latter occurred in patients specifically treated. (d) 157 fluids came from patients with lues, but without any known neurosyphilis, i.e., primary and secondary lues. Of these 150 were negative and 7 were positive.

These results argue favorably for the reliability of our tests. Negative Wassermann reactions were found in fluids from nonluetic lesions and a strong predominance of positive reactions from nervous lesions of known luetic nature. It was evident that such basis for comparison between results of the Wassermann reaction and other spinal fluid reactions must be established before any conclusions can be made.

2. *Cell Count.*—The counting of cells was made in the Fuchs-Rosenthal counting chamber, and the whole volume, 3.2 c.mm. was counted. The cell count per c.mm. was always the one stated, as a third of the total count, such as 7/3 cells per c.mm. representing a total count of 21/3 cells for the 3.2 c.mm. As far as possible, the counting was always made from the first portion of the fluid obtained by the spinal puncture. When blood occurred in the first portion, the counting was made in the subsequent clear portion of fluid. The counting of cells was always repeated twice, partly from the same pipette and partly from different pipettes. An average value was taken of the combined cell counts.

The authors do not propose to fix a limit for the normal cell count of a spinal fluid, but have only considered values between 0/3 and 8/3 as normal ones, and values of 9/3 and above as pathologic. The opinions of various authors differ considerably as to what value should be considered the highest normal cell count. Thus Axel Neel claims 1/3, Jeanselme and Chevalier 4/3, Eskuchen, Holzemann and Pappenheim 15/3 and Nonne 30/3. The figure

probably lies lower than 8/3, but for practical purposes the normal borderline should not be placed too low when dealing with luetic material.

3 and 4. *Globulin and Albumin.*—Globulin and albumin were examined according to the methods described by Bisgaard,[10] Ross and Jones,[11] Boyd,[12] Turner,[13] and Zaloziecki.[14] The total albumin was determined by a disc method on the principle of the Heller nitric acid reaction (28 per cent acid), the spinal fluid being diluted in normal saline solution and the reading being made in the apparatus of Bisgaard[10] after three minutes. A dilution of 15 was considered pathologic, since control tests in normal fluids show that the dilution in these never exceeded 10.

The globulin content was performed in the same manner, using a solution of ammonium sulphate in place of nitric acid. If a qualitative test showed the presence of globulin, the fluid was diluted in saline solution and the result given as the dilution figure. A negative reaction was recorded as 0, and a positive reaction was considered pathologic.

5. *The Colloidal Gold Precipitation Reaction.*—The colloidal gold precipitation was carried out according to the ordinary titrations of 1:10, 1:20, etc., up to 1:10,240. The colloidal gold was developed from 500 c.c. twice distilled water, 5 c.c. of 1 per cent gold chloride solution, 5 c.c. of 2.5 per cent glucose solution and the addition of a 2 per cent solution of potassium carbonate until the color of the colloidal gold was changed. The reactions were recorded in figures from 0 to 5.0. The colloidal precipitation reactions have partly been conducted by the authors and partly by Dr. A. Fölling, of the medical laboratory at the Riks Hospital at Oslo.

To carry out a quantitative comparison between different reactions, it is necessary to obtain a standard, as homogenous as possible, for determination of the degree of the reaction, and difficulty was encountered in finding such a measure. We finally resorted to the use of such terms as "weakly positive," "positive" and "strongly positive" reactions. It is necessary, then, to define what we understand by these terms.

On the subject of pleocytosis it was stated that values below 9/3 were considered normal. Values between 9/3 and 30/3 were considered as "slight pleocytosis" or a "weakly positive" reaction. Those between 31/3 and 100/3 simply as pleocytosis or a "positive" reaction, while all values above 100/3 were considered as "strongly positive" reactions.

In the globulin reaction, the dilution figure 1.0 was considered "weakly positive"; 2.0 "positive" and all values above this figure as strongly positive reactions. For albumin, the same scheme was used, only that the dilution figures for albumin were: 14-25, "weakly positive"; 26-40, "positive"; and all figures above 40, "strongly positive."

The colloidal gold precipitation was recorded as follows: a luetic curve was considered, "weakly positive," a tabetic curve, "positive," and a paralytic curve, "strongly positive."

The Wassermann reaction appeared to best advantage by the use of this scheme since the three dilutions were always employed. It was quite natural to consider a reaction in all three doses as "strongly positive," one positive

in 0.5 c.c. but not in 0.2 c.c. as a "positive" reaction, while those positive only in the large dose of 1.0 c.c. of spinal fluid as "weakly positive" reactions.

In the following tests, a spinal fluid was not considered normal without first showing the following characteristics: Cell count not above 8/3, globulin reaction negative, albumin not above 14, the colloidal gold reaction not above 1 and the Wassermann reaction negative.

Our material thus examined consisted of 69 cases of manifest neurosyphilis and 74 cases of latent syphilis. The 69 cases fell into the following three groups:

General paresis, 18 cases with 21 spinal punctures.
Tabes dorsalis, 18 cases with 28 spinal punctures.
Neurosyphilis of other types, 33 cases with 63 spinal punctures.

The 74 cases of latent syphilis fell into two groups, namely:

Latent syphilis* with normal fluids, 35 cases with 35 spinal punctures.
Latent syphilis* with pathologic fluids, 39 cases with 53 spinal punctures.

TABLE I

RELATIONSHIP BETWEEN THE SPINAL FLUID TESTS AND THE WASSERMANN REACTION

A. AMONG UNTREATED CASES

WASSERMANN REACTION		CELL COUNT	GLOBULIN	ALBUMIN	COLLOIDAL GOLD REACTION				
DOSES IN C.C.	RESULT				CURVES	PER CENT	POS.	NEG.	NUMBER OF FLUIDS
0.1–0.2	++++	424/3	3.5	62	Paralytic	77			
		(++++)	(++++)	(++++)	Tabetic	9	100	0	22
					Lues	14			
					Normal	0			
0.5	+++	234/3	2.7	37	Paralytic	40			
	++	(++++)	(++)	(++)	Tabetic	20	90	10	10
					Lues	30			
					Normal	10			
1.0	+	61/3	0.9	50	Paralytic	6			
		(+)	(−)	(++++)	Tabetic	13	75	25	17
				26*	Lues	56			
				(+)	Normal	25			
−	−	35/3	0.1	11	Paralytic	0			
		(+)	(−)	(−)	Tabetic	4	19	81	72
					Lues	15			
					Normal	81			
B. AMONG TREATED CASES									
0.1–0.2	++++	57/3	1.56	32	Paralytic	48			
		(+)	(+)	(+)	Tabetic	48	100	0	25
					Lues	4			
					Normal	0			
0.5	+++	24/3	0.29	16	Paralytic	9			
1.0	+	(+)	(−)	(+)	Tabetic	52	83	17	24
					Lues	22			
					Normal	17			
−	−	26/3	0	12	Paralytic	8			
		(+)	(−)	(−)	Tabetic	31	77	23	23
					Lues	38			
					Normal	23			

*Corrected and explained in text.

++++ = strongly positive reaction, +++ and ++ = positive, + = weakly positive, and − = negative reaction.

*A distinction between early and advanced cases of latent syphilis does not play any rôle in this study. The only object of our study is to show the correlation of various reactions at the time of examination of the fluid and not to give a continued study of the fluid in the different phases of syphilis.

Thus, altogether, our material consisted of 143 cases with 200 spinal fluids.

By studying the results of the examinations of 200 spinal fluids, a prominent difference was found to exist between the results of the examinations of fluids from untreated and from treated cases, particularly when individual reactions were compared. In Figs. 1 and 2, recording separately the untreated and treated cases, this difference became very conspicuous. In regard to the untreated cases, it was seen that a negative or "weakly positive" Wassermann reaction corresponded to:

1. General low cell count (left in Figs. 1 and 2).
2. Low albumin content and slightly positive colloidal gold reactions (light or very lightly darkened circles, in Figs. 1 and 2).

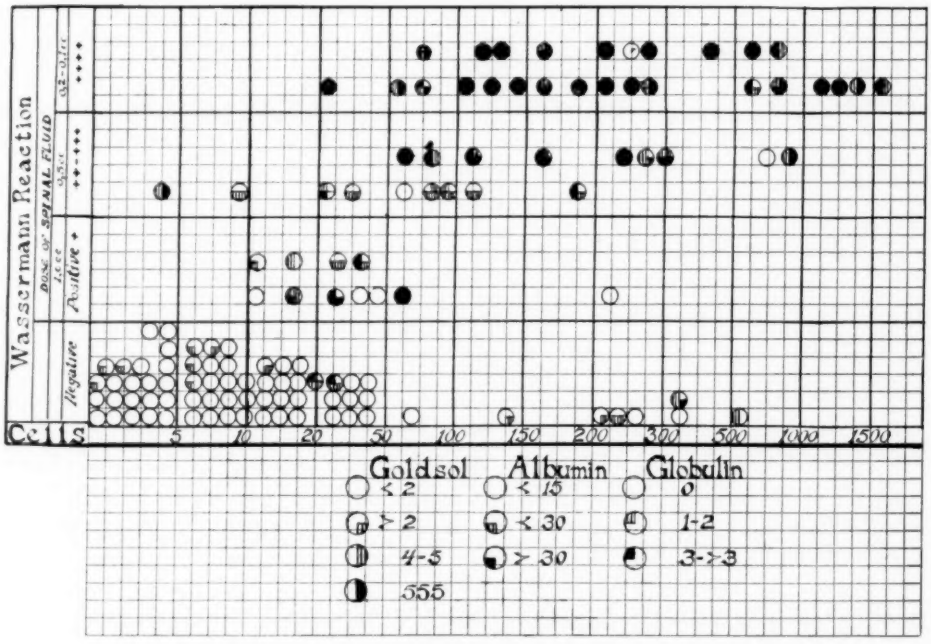

Fig. 1.—Untreated cases.

It was noted in Figs. 1 and 2 that as the circles with an increasing strength of the Wassermann reaction moved upwards along the ordinate, simultaneously was noted a transposition of the circles to the right, i.e., the cell count increased and the circles became more and more darkened, i.e., the globulin (albumin) and the colloidal gold reactions increased in strength.

In order to reduce the above facts into figures, it becomes necessary to find an average value for the results of the examinations of all the fluids lying in the same graphic field. This was done in Table I.

Table I A, dealing with the untreated cases showed that a "strongly positive" Wassermann reaction generally coincided with a very high cell count, strong globulin (albumin) and colloidal gold reactions, while a "positive" and "weakly positive" Wassermann reaction occurred together with lower figures for the other reactions. This agreement between the reactions oc-

curred in 83.2 per cent, while a larger or smaller disagreement was found in 16.8 per cent. To find a reason for the discrepancy between the various reactions was of the greatest concern to the authors in this inquiry. Disagreements between the Wassermann reaction and other reactions will be discussed below.

A positive Wassermann reaction was not found in a single case without the fluid also showing other pathologic reactions. In 49 out of 53 positive cases, at least two of the other reactions were positive. In this connection it must be mentioned that our material did not contain more than two cases of primary lues, while Schou,[8] Fleischmann[15] and other authors found a positive Wassermann reaction in the spinal fluid as the only pathologic reaction.

Considering the relation of the Wassermann reaction to the other reactions and starting with the cell count, we found that a "strongly positive" Wassermann reaction occurred with an average cell count of 424/3, and

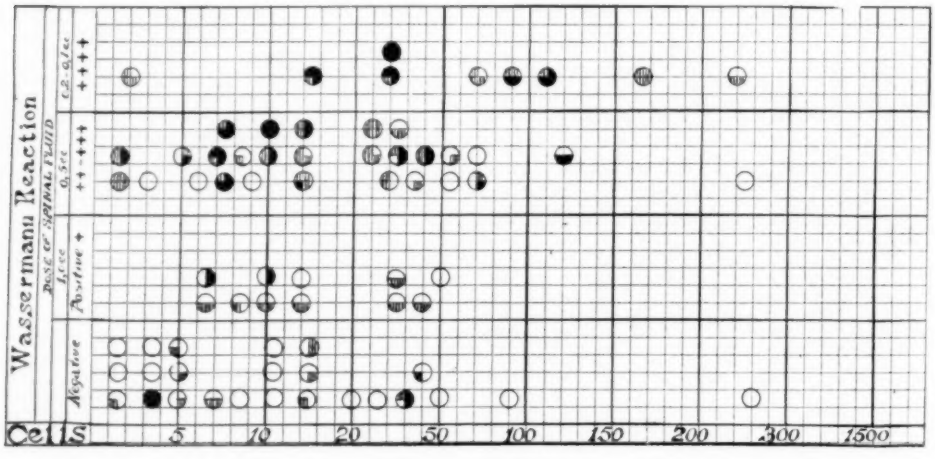

Fig. 2.—Treated cases.

varying at extremes between 1531/3 and 53/3. In a "positive" Wassermann reaction (dose 0.5 c.c.) the average cell count was 234/3, varying at extremes between 510/3 and 64/3, while a "weakly positive" Wassermann reaction was found with a cell count of 61/3, the maximum count being 234/3 and the minimum 8/3. A negative Wassermann reaction corresponded to a cell count of 35/3, the highest figure being 503/3, occurring in a case of transverse myelitis, and the lowest figure 0/3. Only 8 cases were found to have a higher count than 50/3 and in 7 of these no clinical signs of disease were found.

In the normal picture disagreements were sometimes found in excessively high cell counts. When such was the case in combination with a negative Wassermann reaction, it occurred usually in a spinal fluid syphilis without clinical symptoms or signs.

In three cases, however, the cell count was proportionally very low, namely, one case of tabes with 8/3 cells, and two cases of cerebrospinal lues with respectively 12/3 and 26/3 cells. All these cases occurred in combina-

tion with a "slightly positive" Wassermann reaction. In the two last cases, the clinical symptoms were those of meningoradiculitis and hemiplegia with paresis of the oculomotor nerve.

A high cell count without other positive reactions was found with only one exception in pure cerebrospinal fluid lues without clinical symptoms. The isolated pleocytosis cannot, therefore, be of any special importance. The very low cell count, however, was very seldom found together with a positive Wassermann reaction. This occurred now and again so that a low cell count cannot always be taken as an assurance of a negative Wassermann reaction, although under these circumstances as a rule, one may expect a negative Wassermann reaction.

As regards globulin and albumin, the quantitative tests showed continually sinking values as the Wassermann reaction diminished in intensity. Thus, in a "strongly positive" Wassermann reaction, the values were respectively 3.5 and 62; in a "positive" Wassermann reaction, 2.7 and 37; in a "weakly positive" Wassermann reaction, 0.9 and 50; and in a negative Wassermann reaction, 0.1 and 11. The high albumin figure of 50 in a slightly positive Wassermann reaction was found in two cases of subarachnoidal block, with abnormal albumin content. If we dismissed these two irregular cases, the proper figure was 27 instead of 50.

In disagreements between the Wassermann and globulin reactions, we twice encountered negative globulin reactions together with "strongly positive" reactions. One case was a patient with cerebrospinal syphilis occurring seven years after the luetic infection. The Wassermann reaction was strongly positive. The cell count 291/3; globulin and albumin respectively, 1 and 10; while the colloidal gold reaction showed 00121000000. The second case was in the spinal fluid from cerebrospinal syphilis with a positive Wassermann reaction, 510/3 cells, negative globulin, normal albumin and colloidal gold reactions. This patient was a man, sixty years of age, with an uncharacteristic dementia, anisocoria and dissimilar abdominal reflexes.

One might consider this an instance of incipient paralysis or a luetic arteriosclerosis. The cerebrospinal fluid reactions, however, ruled out encephalitis, disseminated, interstitial lesions or any considerable meningeal affection. In two other cases, we likewise found relatively too low values for globulin and albumin when compared with the Wassermann reaction. This picture was obtained in 4 out of 125 fluids.

On the other hand, we found in 5 out of 125 fluids a relatively too high value of the globulin and albumin reactions. This coincidence seemed to be of greater importance than the high cell count, as we have found this condition only in cases of manifest neurosyphilis.

The colloidal gold reaction was never normal in fluids with the strongest positive Wassermann reaction. As was shown in Table I, paralytic curves were found in more than three-fourths of the fluids that gave a "strongly positive" Wassermann reaction. In positive Wassermann reactions, the paralytic curves were found in about 40 per cent of the fluids. On the other hand, the fluids with negative Wassermann reactions never gave paralytic curves, but presented normal curves in 81.5 per cent. The negative Wassermann reactions included

72 fluids of which 3 showed positive colloidal gold reactions, respectively, cerebrospinal lues with 12300000000 and with 02332000000 and hemiplegia with 12300000000. All the other curves were normal or approximately normal.

Treated Cases.—The types and intensities of treatment have raised many questions as to results in spinal fluids, especially since the advent of malaria. It is a subject for another paper and not for this one to try to group results according to the type and duration of treatment.

While a striking parallelism existed between the results of the different reactions of the spinal fluids from untreated cases, the treated ones gave quite a different picture. By "treated" cases we understand cases that have been specifically treated with salvarsan or with bismuth in the course of the last six months previous to our investigation of their spinal fluids. Looking over the graphic scheme of the treated cases, we found that the transposition to the right side with the increasing strength of the Wassermann reaction is not quite as characteristic as among the untreated cases. The cell count was low as compared to the strength of the Wassermann reaction. By calculating the average values of the cell counts as mentioned above, we found a very prominent difference between the two kinds of patients. This was easily seen from Table I B. Corresponding to a "strongly positive" Wassermann reaction, we thus found only 57/3 cells against 424/3 in untreated cases, while the "positive" and "weakly positive" Wassermann reactions taken together corresponded to 24/3 cells and the untreated ones showed 234/3 and 61/3 separately.

The globulin and albumin figures likewise showed much lower values than did the same reactions in the untreated cases, which was easily seen from Table I B.

The colloidal gold reaction, however, presented a different picture, inasmuch as the fluids with a negative Wassermann reaction showed many pathologic curves. When the Wassermann reactions of medium strength were compared with the cell count and globulin-albumin reactions, there was likewise found a considerable number of pathologic curves. Among the Wassermann reactions of medium positive strengths, the positive colloidal gold curves were practically as numerous as among the untreated cases. This was easily seen from Table II, where the colloidal gold curves were tabulated.

Comparing the colloidal gold reaction curves with negative Wassermann reactions, a very prominent difference was found of 19.5 per cent positive curves in untreated cases, against 76.87 per cent positive curves in treated patients. Among these some were paralysis curves and about the same proportion of tabes and lues curves.

TABLE II

CORRELATION OF WASSERMANN REACTION AND COLLOIDAL GOLD PRECIPITATION REACTION

WASSERMANN REACTION	PER CENT POSITIVE COLLOIDAL GOLD REACTIONS	
	UNTREATED CASES	TREATED CASES
Strongly positive 0.2 c.c.	100	100
Positive 0.5 c.c.	90	83
Slightly positive 1.0 c.c.	75 — 81.4	
Negative	19.5	76.87

Thus our material showed very distinctly that while the cell count and globulin-albumin reactions were influenced in a relatively high degree by the treatment, the Wassermann reactions and colloidal gold reactions were very resistant, the latter especially being the most resistant to the course of anti-syphilitic treatment.

SUMMARY

Among 200 spinal fluids from syphilis, neurosyphilis, spinal fluid syphilis and latent syphilis, a considerable difference between treated and untreated cases was observed by a study of the ordinary spinal fluid reactions, such as the cell count, globulin and albumin reactions, the Wassermann and colloidal gold reactions.

1. Among the untreated cases, 83.2 per cent showed a consistent agreement between the above-mentioned reactions as regards the quality and degree of the reactions. The remaining 16.8 per cent of the fluids showed a larger or smaller disagreement between the various reactions. The causes of such disagreement were:

a. Most frequently a relatively too high cell count. This occurred in 8 out of 125 cases. In 7 of these the clinical diagnosis was the so-called "spinal fluid syphilis" which may be due to an increase of lymphocytes in the cerebrospinal fluid.

b. Less frequently it may be accompanied by a relatively high albumin content. The source of the 5 fluids wherein this condition was found, were cases of manifest neurosyphilis.

The colloidal gold reaction in no case occurred as an isolated pathologic reaction.

The Wassermann reaction was in no case found to be positive without one or several other reactions being pathologic. Among the 53 fluids with a positive Wassermann reaction, only 4 showed one single pathologic reaction beside the Wassermann reaction, while the 49 others showed several pathologic reactions.

In cases with strongly positive, positive and weakly positive Wassermann reactions, the cell counts were respectively 424, 234, 61 and with a negative Wassermann reaction, 35 cells. In one single case of tabes, a positive Wassermann reaction was found combined with a normal cell count. On the other hand, 503 cells were found in a case with a negative Wassermann reaction in a male suffering from a transverse myelitis.

2. In 75 spinal fluids from treated cases, agreement between the reactions was observed in only 34.67 per cent. In the other 65.33 per cent, there was a more or less pronounced disagreement.

a. This disagreement was mostly caused by the low or very low cell count and albumin, simultaneously with a positive or strongly positive Wassermann reaction or colloidal gold reaction. Table I B showed that the various degrees of the positive Wassermann reactions corresponded to very much lower cell counts, globulin and albumin content following the treatment rather than before treatment was started. The colloidal gold reaction showed the same amount of positive curves before as well as after treatment, although a greater

amount of strongly positive reactions were found after treatment, when evaluated according to the results of the Wassermann reactions. In the presence of a negative Wassermann reaction, we found a greater number of positive reactions of the colloidal gold after treatment than before.

b. In fluids from treated cases, the Wassermann and colloidal gold reactions may occur as isolated pathologic reactions individually or combined, while all other reactions may be completely negative. A positive Wassermann reaction was thus found twice in fluids from treated cases; otherwise the spinal fluid was completely normal. On the other hand, this combination was never observed among untreated cases.

In conclusion it may be said that the final criterion signifying that a spinal fluid in neurosyphilis has become normal during treatment, must be either a negative Wassermann or colloidal gold reaction, or better still, these two reactions occurring negative together. The finding of a negative globulin reaction, a normal cell count and albumin, will not suffice, however, as a means of telling whether or not a spinal fluid from a luetic is normal.

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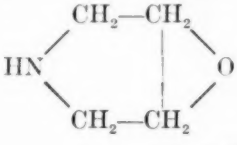
STUDIES IN TOXICOLOGIC CHEMISTRY*

II. The Formaldehyde-Sulphuric Acid Reaction of the Opium Alkaloids

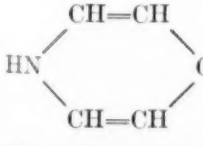
By Victor E. Levine, M.D., and Estelle A. Magiera, M.D., Omaha, Nebraska

THE chemical properties of the morphine molecule, its derivatives and the alkaloids closely associated with it, are interesting in view of the fact that these properties furnish the underlying reasons for many of the procedures at present employed in toxicology for their identification. Most of the tests for morphine and its derivatives relate to three of their conspicuous properties. One of these is their great avidity for oxygen. The other lies in their ability to lose a molecule of water on treatment with a dehydrating agent like hydrogen chloride or concentrated sulphuric acid. The resulting compound is apomorphine, which is morphine deprived of a molecule of water. The formation of this alkaloid is responsible for some very characteristic color reactions given by morphine, codeine, and heroin. The third notable property of morphine and its associates is their reactivity relating to the presence of the phenolic group.

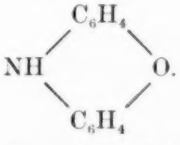
Morphine readily reacts with oxygen. Oxidation can be accomplished by atmospheric oxygen in the presence of alkali, as well as by nitrous acid, potassium ferricyanide, or ammonium copper sulphate. The first product of oxidation is oxydimorphine or pseudomorphine $(C_7H_{18}NO_3)_2$. This compound is non-toxic and is produced in the organism as a detoxication product of morphine. Further oxidation results in the formation of morpholin and fragments belonging to phenanthrene, and possibly derivatives of naphthalene. These decomposition products possess chromogenic properties. Morpholin, the formula of which is



is related to oxazine,



and to the phenoxazine



The latter forms the nucleus of well-known blue and violet coal tar dyes of determined constitution. The synthetic product, which Chastaing named morpholin blue, is an example of one of the color compounds closely allied to morphine.[1]

The ease of formation of chromogenic substances from morphine and its allied alkaloids explains why in their presence many oxidizing agents yield characteristic color reactions. Chlorine water colors morphine yellowish; chlo-

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rine gas yields a deep yellow; ammonium hydroxide changes the color to red or brown. A solution of iodic acid in sulphuric acid colors morphine dark violet, then brown (Selmi[2]). When morphine is heated with concentrated sulphuric acid and a drop of a solution of potassium chlorate subsequently added, a fine and long-enduring grass-green color is produced, while the borders of the liquid become faintly rose-red (Donath[3]). Mixing morphine with sulphuric acid and sodium arsenite results in the production of a dirty violet color changing to dark sea-green (Tattersall[4]). Morphine dissolved in dilute sulphuric acid yields a pale rose color with lead dioxide. Addition to the filtrate of ammonium hydroxide in excess produces a brown color, which persists for several hours. The color becomes transitorily dark gray when the mixture is heated until white fumes are evolved. Morphine also reacts with nitric acid, and with a mixture of sulphuric and nitric acids (Husemann's reaction[5]). We shall consider later the reaction of nitric acid with relation to the phenolic properties of the opium alkaloids.

All substances that are readily oxidized have the ability to reduce compounds that give up oxygen with ease. That morphine is a great reducing agent is evident from the fact that it readily reduces gold and silver compounds even in the cold. In concentrated sulphuric acid it also reduces bismuth subnitrate with the production of a dark brown color.

One of the tests for morphine depends upon its behavior toward iodic acid. This latter compound is reduced to hydriodic acid. This acid easily decomposes in the presence of inorganic acid with the liberation of free iodine, which turns starch blue. The liberation of iodine from iodic acid is brought about promptly by many reducing agents, both organic and inorganic. The response of morphine to iodic acid is, therefore, not distinctive of this alkaloid. It is but an illustration of its reducing ability.

According to Lefort,[6] the use of ammonium hydroxide after the addition of iodic acid results in the formation of a characteristic mahogany color. The ammonium hydroxide decolorizes the liberated iodine, and therefore permits the development of the color of the oxidation products of morphine. Lefort's test is believed to be distinctive of morphine and is regarded as having a positive value in proof of its presence. The iodic test without ammonium hydroxide has only a negative value. However, failure of reduction with the failure of formation of free iodine is proof of the absence of morphine.

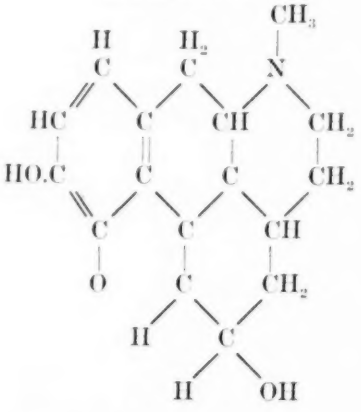
Morphine also reacts with a mixture of ferric chloride and potassium ferricyanide. The alkaloid brings about the formation of a blue color, the product resulting being ferrous-ferricyanide or ferric-ferricyanide or perhaps a mixture of both. Levine[7] has recently pointed out that many organic compounds, especially phenols, reduce the ferric-ferricyanide reagent. However, the test has a negative value in establishing the absence of morphine, other reducing substances being excluded.

Another test based upon reduction is that involving the use of Fröhde's reagent[8] (molybdic acid in concentrated sulphuric acid) or Buckingham's reagent (sodium molybdate in concentrated sulphuric acid). Morphine is oxidized, while the molybdic acid is reduced, giving rise to compounds yielding colored solutions. The color produced with morphine is deep purple,

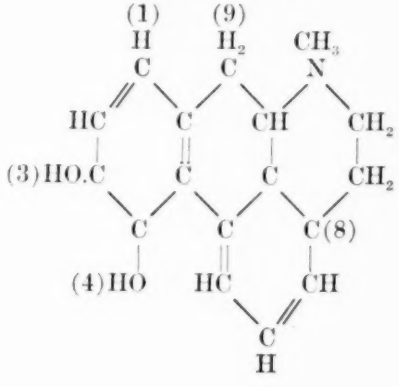
fading to violet, then changing to green. Levine and Jahr[9] have shown that a reagent similar to Fröhde's or Buckingham's has a wide range of reactivity, giving positive results with aldehydes, ketones, carbohydrates, amino acids, proteins, and other compounds. Fröhde's reagent gives bluish colors with codeine and narcein, greenish with apomorphine and berberine, and reddish shades with brucine, emetine (changing to green), and yellowish tints with veratrine. In connection with the reduction of molybdenum compounds, it may be added that the precipitate of morphine obtained by means of phosphomolybdic acid dissolves in ammonium hydroxide with a blue color.

We now come to those tests bearing on the conversion of morphine into apomorphine. Solutions of the latter alkaloid assume a blue color when shaken with alkali. When morphine is converted into apomorphine, this color reaction with alkali develops (Denigés[10]). Grimbert and Leclère[11] have shown that the same reaction may be produced with greater rapidity and intensity by boiling an apomorphine solution with sodium acetate and mercuric chloride. Denigés extended this reaction to morphine. A few milligrams of morphine with two or three drops of concentrated sulphuric acid are added to con-

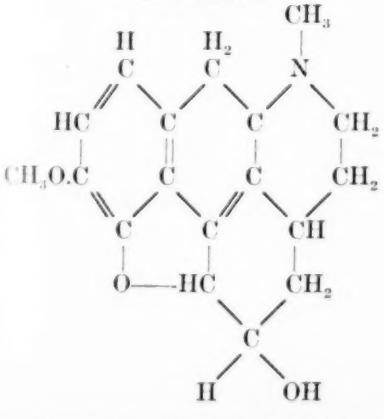
MORPHINE



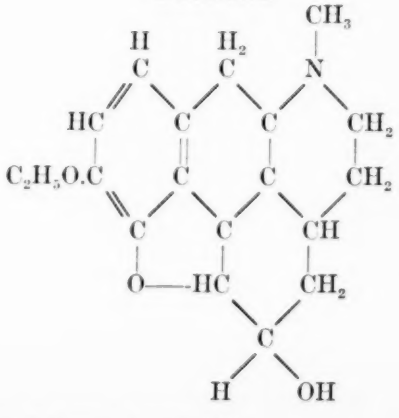
APOMORPHINE



CODEINE



DIONINE

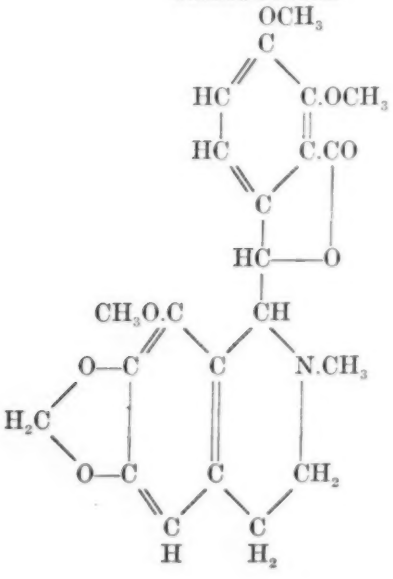


Heroin is diacetyl morphine; peronine is benzyl morphine.

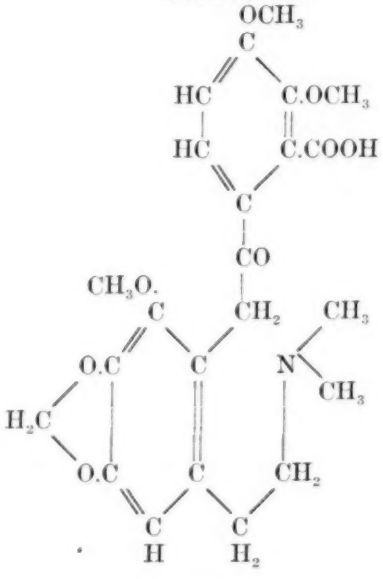
vert the morphine into apomorphine. The mixture is now diluted with 5 c.c. of a saturated solution of sodium acetate and two drops of 4 or 5 per cent solution of mercuric chloride. The whole mixture is boiled. Other alkaloids which can be converted to apomorphine—codeine, heroin and dionine—also respond to the test.

In the Pellagri reaction the alkaloid is first dissolved in fuming hydrochloric acid, concentrated sulphuric acid is next added, and the final mixture evaporated on an oil bath at 100° C. to 120° C. Apomorphine, morphine, codeine, and heroin show purple at the edges. After the evaporation of the

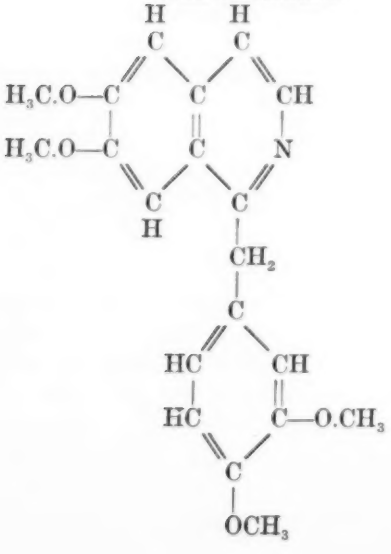
NARCOTINE



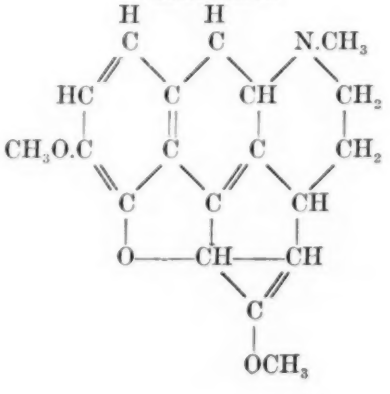
NARCEINE



PAPAVERINE



THEBAINE



hydrochloric acid, the purple assumes a red color. The latter changes to violet on re-addition of hydrochloric acid followed by a neutralization with sodium bicarbonate. If hydriodic acid or a dilute solution of iodine in alcohol is now added, the color changes to green and dissolves in ether, which it tints purple.

Morphine possesses three oxygen atoms. One is alcoholic, another is phenolic, and the third is indifferent, forming the so-called bridge-oxygen atom. Codeine is a phenolic ether, methyl morphine. Dionine is ethyl morphine. Heroin is diacetyl morphine; peronine is benzyl morphine. Apomorphine is made from morphine by the removal of a molecule of water as a result of dehydration with concentrated hydrochloric acid. It has two phenolic hydroxyls. Thebaine has two methoxy groups; narcotine and narceine, three; papaverine, four. The formulas for the various alkaloids are given on pp. 775 and 776.

Certain tests for morphine are directly referable to the presence in its molecule of the phenol group. Ferric chloride gives characteristic color reactions with many phenols. With this iron salt morphine yields a blue color, which is due to the reduction of the ferric salt to the ferrous state and to the oxidation of morphine. It is very likely that the main color product is a substance closely bound up with the phenol group. It is to be remembered that phenols other than morphine—gallic, tannic, and salicylic acids—also yield blue colors with ferric chloride.

The reaction with nitric acid or with sulphuric and nitric acids is typical of phenols. Concentrated nitric acid dissolves morphine with an orange red or deep red color, finally changing to yellow. The yellow color is due to reactions analogous to those taking place in the formation of yellow picric acid or trinitrophenol from phenol. In the Husemann test morphine is dissolved upon a watch glass in a few mills of concentrated sulphuric acid. The colorless solution is heated for thirty minutes on the water-bath, until white fumes arise. The mixture is allowed to cool, and two or three drops of concentrated nitric acid are added. A fugitive, reddish-violet color appears, soon changing to blood red, yellowish-red or yellow, and gradually disappearing. Potassium nitrate may be substituted for nitric acid. Oxydimorphine, apomorphine, codeine, and heroin react like morphine. Levine and Hammill[13] have shown that monophenols, diphenols, triphenols, and also derivatives of these types, react with nitric acid in the presence of sulphuric or phosphoric acid to give, as a rule, a red or reddish-purple color.

Uranium nitrate (5 per cent) in alcohol or aqueous solution and neutralized with ammonium hydroxide gives, according to Lamal,[14] with morphine in not too dilute a solution, an orange or red color. Phenol with uranium acetate also yields a red color (Orlow[15]).

Still another test which seems to depend upon the presence of the phenolic group is Lautenschlager's diazonium test.[16] Morphine readily forms dyestuffs with diazonium compounds. A red color is produced on the addition of diazotized sulphanilic acid to morphine in alkaline medium. Under the same conditions sparteine yields a yellowish color; coniine and nicotine, a bright yellow; emetine and physostigmine, a red color. Of the alkaloids that

react at all with the Lautenschlager procedure, only morphine is stable in acid solution, although the red color formed changes to orange on the addition of acid. The fact that no other opium alkaloid except morphine reacts to Lautenschlager's reagent leads to the belief that the free phenolic group is cause for the positive response, since no such free group exists in codeine (methyl morphine), dionin (ethyl morphine), heroin (diacetyl morphine), narcein, narcotine, thebaine, or papaverine.

Very recently another test has been added to the long list of tests already known for the morphine alkaloids. Ekkert[16a] in 1926 described a color reaction for morphine based upon condensation with benzidine in presence of concentrated sulphuric acid. The test proved positive for morphine, methyl morphine, ethyl morphine, diacetyl morphine, benzyl morphine and apomorphine. Experiments, which we will report later, seem to indicate that the same reagents react also with non-alkaloidal phenols.

THE REACTION SYSTEM, PHENOL-ALDEHYDE-ACID

Many color reactions employed in biochemistry result from the interaction in the system, phenol-aldehyde-acid. The phenol and aldehyde condense to form a colored product. By the use of this system, characteristic tests may be obtained for any one of three components in the system: (1) aldehydes or compounds yielding aldehydes on oxidation (alcohols, such as methyl alcohol and glycerol) or on decomposition (carbohydrates, aliphatic carboxylic acids, such as oxalic, succinic, malic, tartaric, citric, glycollic, and lactic acids); (2) phenols of all types; (3) inorganic acids, such as hydrochloric or sulphuric acid. Chloroform, bromoform, and iodoform can also be tested for by this triple component system, for these halogen derivatives react readily with sodium or potassium hydroxide to yield the sodium or potassium salt of formic acid. This acid is a carboxylic acid which may be regarded as also possessing an aldehyde grouping.

TEST FOR ALDEHYDES

To test for aldehydes some of the well-known reagents used consist of a phenol and an inorganic acid possessing dehydrating properties like hydrochloric or sulphuric. The reagents, phenol and a dehydrating acid, together with the aldehyde-containing substance to be tested, complete the reaction system, phenol-aldehyde-acid.

The table gives a few tests for formaldehyde. These tests can also be employed for the detection of methyl alcohol, when on oxidation it is made to yield formaldehyde.

TABLE I—FORMALDEHYDE

PHENOL	ACID	END-RESULT
Phenol	H_2SO_4	Red ring
Resorcin	H_2SO_4	Red color
Naphthoresorcin	HCl	Flocculent precipitate, darkening on standing
Guaiacol	H_2SO_4 and $FeSO_4$	Violet ring
Phloroglucin	HCl	Finely divided precipitate, solution becoming orange in color
Pyrogallol	H_2SO_4	Chocolate brown color
Gallic acid	H_2SO_4	Green ring, changing to deep blue ring

TESTS FOR GLYCEROL

The system, phenol-aldehyde-acid, can be employed for the detection of glycerol. When this trihydric alcohol is oxidized by chlorine or bromine, it yields dihydroxyacetone and eventually the aldehyde, methyl glyoxal. After oxidizing the glycerol, the excess of bromine or chlorine is driven off. An alcoholic solution of orcinol, resorcinol or codeine is used together with concentrated sulphuric acid to give the reactions shown in the table.

TABLE II—GLYCEROL

PHENOL	ACID	END-RESULT
Orcinol	H_2SO_4	Beautiful violet or greenish-blue
Resorcinol	H_2SO_4	Wine-red color
Codeine	H_2SO_4	Beautiful greenish-blue on heating

In the Mandel-Neuberg[16b] test for glycerol oxidation is brought about by means of sodium hypochlorite. The aldehyde, glycerose, which is formed, is made to react with orcinol in the presence of hydrochloric acid to give a beautiful violet or green-blue color.

TESTS FOR ALIPHATIC CARBOXYLIC ACIDS

The following acids—oxalic, citric, succinic, tartaric, malic, glycollic, lactic—decompose on treatment with strong sulphuric acid to yield formaldehyde or the aldehyde acid, formic acid, which decomposes to form carbon monoxide and water. Owing to their characteristic decomposition these acids readily respond to tests with reagents containing a phenol and concentrated sulphuric or hydrochloric acid.[16c] The table following illustrates the reaction system, phenol-aldehyde-acid, applied to the testing of some aliphatic carboxylic acids.

TABLE III—ALIPHATIC CARBOXYLIC ACID

ALDEHYDE-PRODUCING ACID	PHENOL	INORGANIC ACID	END-RESULT
Succinic acid	Resorcin	H_2SO_4	Yellowish red solution with green fluorescence
Malic acid	β-naphthol	H_2SO_4	Blue color, changing to green on heating
Tartaric acid	β-naphthol	H_2SO_4	Intense blue color
Tartaric acid	Resorcin	H_2SO_4	Bright red color
Tartaric acid	Pyrogallol	H_2SO_4	Fine violet blue color
Citric acid	β-naphthol	H_2SO_4	Greenish yellow, changing to bright yellow on heating

Since oxalic acid decomposes on treatment with concentrated sulphuric to form the aldehyde acid, formic acid, it also responds to reactions resulting from its interaction with sulphuric acid and a phenol. According to Denigés,[16d] p-cresol, guaiacol, and codeine can be used as reagents for the detection of glycollic or lactic acid. Glycollic acid decomposes on treatment with sulphuric acid to yield formaldehyde, while lactic acid under similar treatment yields acetaldehyde and formic acid.

TESTS FOR CHLOROFORM

Chloroform, bromoform, and iodoform react with potassium hydroxide to form potassium formate. Formic acid in its structural composition contains an aldehyde grouping. This fact argues for the possibility of utilizing the

system, phenol-aldehyde-acid, as a means of detecting the halogen compounds mentioned. That this is the case is evident from the Schwarz resorcinol[16e] test and the Lustgarten naphthol test for chloroform, bromoform or iodoform.

TABLE IV—CHLOROFORM

NAME OF REACTION	PHENOL	ALKALI	END-RESULT
Schwarz	Resorcinol	NaOH	Yellowish red color attended by a beautiful yellowish fluorescence
Lustgarten	α- or β-naphthol	KOH	Evanescent blue color, changing in contact with air to green, then to brown.

In the above tests alkali is used instead of acid. The alkali is used to decompose the trihalogen derivatives. That acid can take part in the reaction is shown by the fact that the blue solution obtained with Lustgarten's reagents can, on acidification, be converted into a red dyestuff.

TESTS FOR FURFURAL

Furfural is an aldehyde obtained on the decomposition of carbohydrates with hydrochloric or sulphuric acid. The tests in the table following illustrate the use of the system, phenol-aldehyde-acid, for the purpose of detecting furfural.

TABLE V—FURFURAL

NAME OF REACTION	PHENOL	ACID	END-REACTION
Molisch	α-naphthol	H_2SO_4	Reddish violet zone
Bial	Orcinol	HCl	Green solution or a green flocculent precipitate

TESTS FOR CARBOHYDRATES

Inorganic acid, sulphuric or hydrochloric acid, and a phenol are reagents used to test for carbohydrates. The acid reacts with the carbohydrate to yield furfural or a derivative thereof. The formation of the aldehyde, furfural, completes the reaction system, phenol-aldehyde-acid.

TABLE VI—CARBOHYDRATES

NAME OF REACTION	TEST FOR	PHENOL	ACID	END-RESULT
Molisch	All carbohydrates, glucolipins and gluco-proteins	α-Naphthol	H_2SO_4	Reddish-violet zone
Seliwanoff	Ketone sugars	Resorcin	HCl	Red color and separation of brown-red precipitate on heating
Tollens	Glycuronates	β-Naphtho-resorcin	HCl	After heating, mixture extracted with ether, which assumes a violet-red color
Tollens	Pentose, galactose, glycuronates	Phloroglucine	HCl	Red color
Bial	Pentose	Orcinol	HCl and $FeCl_3$	Green color and a green flocculent precipitate

TESTS FOR FREE INORGANIC ACID IN GASTRIC CONTENTS

The reaction system, phenol-aldehyde-acid, is a useful one in detecting inorganic acid, like hydrochloric or sulphuric. Boas' test and Günzberg's test

serve to detect free hydrochloric acid in gastric contents. The reagents used, phenol and aldehyde, complete the triple system, phenol-aldehyde-acid.

TABLE VII—FREE ACID IN GASTRIC CONTENTS

NAME OF REACTION	PHENOL	ALDEHYDE	END-RESULT
Boas	Resorcinol	Cane sugar (yielding furfural with acid)	Rose-red color after heating
Günzberg	Phloroglucin	Vanillin	Purplish-red color after heating

TESTS FOR PHENOL

For identifying phenols, it is necessary to use a mixture of aldehyde and acid. Melzer's reaction[17] for phenol, Mörner's reaction for tyrosine, a phenolic amino acid, Pettenkofer's[18] or Udránsky's[19] reaction for morphine are illustrations of the system, phenol-aldehyde-acid, applied to the qualitative analysis of phenols.

TABLE VIII—PHENOLS

NAME OF REACTION	TEST FOR	ALDEHYDE	ACID	END-RESULT
Melzer	Phenol	Benzaldehyde	H_2SO_4	Violet-blue color
Mörner	Tyrosine	Formaldehyde	H_2SO_4	Green
Udránsky	Morphine	Furfural	H_2SO_4	Purple, changing to blood red
Pettenkofer	Morphine	Sucrose (yielding furfural with acid)	H_2SO_4	Purple, changing to blood red
	Oxydimorphine	Sucrose	H_2SO_4	Green, changing to blood red
	Codeine	Sucrose	H_2SO_4	Purple, changing to blood red

TESTS FOR PHENOLIC ALKALOIDS

In 1896, Marquis[20] first reported his test for the phenolic alkaloids, depending upon the color formed with formaldehyde in the presence of sulphuric acid. The Marquis test seems to fit in with the scheme of the phenol-aldehyde-acid reaction. The phenols are the opium alkaloids, while the aldehyde and the acid are contained in the reagent.

TABLE IX—PHENOLIC ALKALOIDS

PHENOLIC ALKALOID	ALDEHYDE	ACID	END-RESULT
Morphine	Formaldehyde	H_2SO_4	Purplish-red, changing to violet, and finally becoming blue
Oxydimorphine	Formaldehyde	H_2SO_4	Green
Codeine	Formaldehyde	H_2SO_4	Violet
Apomorphine	Formaldehyde	H_2SO_4	Violet
Narcotine	Formaldehyde	H_2SO_4	Olive green, changing to yellow

PHENOL-ALDEHYDE-SULPHURIC ACID REACTIONS

MONOPHENOLS

Phenol ----------raspberry red.
o-Cresol ----------deep purple; deep red; dark brown.
(methyl phenol)
m-Cresol ----------deep purple; deep red.
p-Cresol ----------deep purple.
o-Xylenol ----------greenish edges; dark red-brown next day.
(dimethyl phenol)

m-Xylenol ---------- reddish brown; changing in a few minutes to brown; in one-half hour changed to olive green which persisted after a week.
p-Xylenol ---------- bright red or crimson changing soon to purple.
Thymol ---------- reddish violet.
Carvacrol ---------- reddish brown; slight tinge of brown around edges.
α-Naphthol ---------- slight reaction; very slight green, with brown tint predominating; violet purple cast also seen in the reaction mixture.
β-Naphthol ---------- olive changing to dark green.

MONOPHENOLS WITH HALOGEN

Tribromphenol ---------- light red.
Di-iodothymol ---------- negative.

NITRATED MONOPHENOLS

o-Nitrophenol ---------- negative.
p-Nitrophenol ---------- negative.
Dinitrophenol ---------- negative.
Picric acid ---------- negative.
(dinitrophenol)
Picramic acid ---------- negative.
(dinitro aminophenol)

MONOPHENOLS WITH AMINO GROUP

Amidol ---------- dark blue.
(1.3-diamino-4-hydroxybenzene dihydrochloride)
Metol ---------- slight violet coloration; slow reaction.
(mono-methyl-p-amido-metacresol sulphate)
Photol ---------- negative.
(mono-methyl p-amido phenol sulphate)

ETHERS OF MONOPHENOLS

Anisol ---------- purplish red.
(methyl phenyl ether)
Phenetole ---------- deep rose.
(ethyl phenyl ether)
Phenacetin ---------- negative.
(acetyl derivative of p-aminophenetole)

MONOPHENOLS WITH ALCOHOL GROUP

Diathesin ---------- not soluble in reaction mixture; solid particles of the compound assume a lavender violet color, which persists even after two weeks.
(o-hydroxy benzyl alcohol)

MONOPHENOLS WITH ALDEHYDE GROUP

Salicylic aldehyde ---------- blood red.
(o-hydroxybenzaldehyde)

MONOPHENOLS WITH CARBOXYL GROUP

Salicylic acid ---------- carmine, lasting for more than three days.
(o-hydroxybenzoic acid)
Aspirin ---------- carmine, very slowly changing to bluish.
(acetyl salicylic acid)

β-Oxynaphthoic acid ---------green.
Tyrosine ---------slow reaction, persistent characteristic deep blue developing
(p-hydroxy phenyl amino- after long standing.
propionic acid)

MONOPHENOLIC ETHERS WITH CARBOXYL GROUP

Anisic acid ---------light green with lavender border.
(p-methoxybenzoic acid)

ESTERS AND SALTS OF PHENOLIC ACIDS

Sodium sulphocarbolate ------pink and red.
(sodium phenol sulphonate)
Zinc sulphocarbolate ---------purplish red, changing to green which persists for several days.
(zinc phenol sulphonate)
Sodium salicylate ----------deep purplish red or carmine.
(oil of wintergreen)
Methyl salicylate ----------poor reaction; evanescent purplish red streak in the yellowish
reaction mixture.
Salol ----------bright purplish red.
(phenyl salicylate)
p-Cresalol ---------purplish red, greenish around edges; after one-half hour
(p-cresol salicylate) changes to persistent green.

DIPHENOLS

Pyrocatechol ---------violet.
(o-dihydroxybenzene)
Adrenalin ---------violet with a brown cast.
(o-dihydroxyphenyl
hydroxyethyl-methylamine)
Orcinol ----------yellow, changing at once to a bright raspberry red, which re-
mains even at the end of three days.
Hydroquinone ---------red brown with green tinge, turning to muddy brown.
(p-dihydroxybenzene)

ETHERS OF DIPHENOLS

Guaiacol ---------purple
(monomethyl ether of
pyrocatechol)
Eugenol ----------dark reddish violet.
(allyl 4, 3 guaiacol)

ETHERS OF DIPHENOLS WITH ALDEHYDE GROUP

Vanillin ---------lemon yellow, changing to brownish red with purple green
(m-methoxy-p-hydroxy fluorescence.
benzaldehyde)
Piperonal ---------light olive green.
(also called heliotropine)
(anhydride of vanillin)

ETHERS OF DIPHENOLS WITH CARBOXYL GROUP

Vanillic acid ---------deep orange red.
(m-methoxy p-hydroxy
benzoic acid)

TRIPHENOLS

Pyrogallol ---------Indian red turning quickly to red brown.
(1, 2, 3 trihydroxybenzol)
Phloroglucinol ---------bright blood orange.
(1, 3, 5 trihydroxybenzol)

TRIPHENOLS WITH CARBOXYL GROUP

Gallic acid (3, 4, 5 trihydroxy benzoic acid)	purplish red changing to greenish, then to deep olive green.
Tannic acid (digallic acid)	evanescent yellowish green; poor reaction.

SALTS OF TRIPHENOLIC ACIDS

Dermatol (bismuth subgallate)	olive green changing to persistent dark green.

GLUCOSIDES YIELDING PHENOLS ON HYDROLYSIS

Arbutin (yields hydroquinone on hydrolysis)	slight green immediately changing to brown.
Salicin (yields saligenin or o-hydroxybenzyl alcohol on hydrolysis)	purplish red, becoming blackish red on standing; with sulphuric acid alone—cherry red, becoming blackish brown on standing.
Aesculin	persistent yellowish green.

PHENOLIC ALKALOIDS

Morphine sulphate	purple red changing to violet and finally becoming blue.
Oxydimorphine	green.
Codeine sulphate (methyl morphine)	violet.
Dionin (ethylmorphine)	violet, changing to deep blue.
Heroin (acetylmorphine)	violet.
Apomorphine	violet.
Cotarnine	purplish red.
Narcotine	olive green, changing finally to yellow.

BITTER PRINCIPLES

Aloin	canary yellow, changing to red brown; same reaction with sulphuric acid alone.
Crysarobin	with sulphuric acid red, which on standing changes to blackish brown; with reagent dark red, which on standing becomes purplish violet.

COMPOUNDS OTHER THAN PHENOLS

α-Naphthylamine	olive, changing to deep green.
β-Naphthylamine	very beautiful intense blue.

The following compounds reacted negatively with the formaldehyde-sulphuric acid reagent:

Aldehydes.—Paraformaldehyde, acetaldehyde, trichloracetaldehyde, benzaldehyde, o-nitrobenzaldehyde, p-dimethylaminobenzaldehyde, cinnamic aldehyde.

Ketones.—Acetone, chloroacetophenone, Michler's ketone.

Alcohols.—Methyl alcohol, ethyl alcohol, amyl alcohol, caprylic alcohol, glycerol, mannitol.

Carboxylic Acids.—Formic acid, acetic acid, butyric acid, caprylic acid, succinic acid, lactic acid, pyromucic acid, diacetic acid, palmitic acid, oleic acid.

Amino Acids.—Glycocoll, alanine, leucine, cystine, tryptophane, aspartic acid, asparagine.

Proteins.—Peptone, gelatin, egg albumin, collagen, osseomucoid.

Carbohydrates.—Xylose, rhamnose, fructose, glucose, mannose, maltose, lacrose, sucrose, trehalose, raffinose.

Lipins.—Olein, palmitin, stearin, lecithin, cholesterol.

Miscellaneous Compounds.—Benzene, m-dinitrobenzene, dimethyl aminobenzene, diphenylmethane, aniline, dimethylamine, p-phenylene-diamine, benzidine, semicarbazide, hydroxylamine, antipyrine, urea, thiourea, creatine, creatinine, uric acid, iodoform, barbital, santonin, sulphonal, trional, pilocarpine, cocaine, quinine, cinchonine, strychnine.

The Marquis reaction is believed to be quite distinctive for the opium alkaloids and is regarded by Kobert[21] as the best at our disposal. Gauss[22] devised a colorimetric method for the estimation of morphine based upon this reaction.

By reason of the fact that the reaction is of the phenol-aldehyde-acid type, there arises the possibility of extending the Marquis procedure to all phenols. We have tested out the hypothesis, and find that the test is applicable to all phenols examined—monophenols, aminophenols, phenol ethers, phenols containing an alcohol, aldehyde or carboxyl group, salts and esters of phenolic acids, diphenols, ethers of diphenols, triphenols, glucosides, and phenolic alkaloids. The detailed results are given in tabular form.

The colorations produced differ in most cases entirely from the phenolic alkaloids. Some phenols of biologic occurrence, however, produce effects similar to those given by these alkaloids. The reactions obtained with pyrocatechin and para-cresol are so similar to those of the opium alkaloids that it is impossible to make a distinction between them. This fact argues for extra caution in interpretation of results. It also detracts from the usefulness of the Gauss method for the colorimetric determination of morphine. The method would be serviceable for the alkaloid in its pure form only, but in toxicologic mixtures or extracts it would be necessary to remove any phenols that react similarly to the alkaloids, or any phenols that would throw off the color given by morphine. For the sake of quantitative exactness it would also be necessary to remove any phenol, or any phenols that would modify the color given by morphine. When a mixture of a phenol and morphine is made, the color yielded with the Marquis reagent is a resultant of that given by the phenol and the alkaloid. The color formed is not comparable to that given by pure morphine, thus impairing the accuracy of the colorimetric procedure devised by Gauss.

Since the color tests for morphine and its associates lack specificity, they should be used with great precaution as evidence of their presence. Wormley[23] recovered morphine from the urine of opium patients, from the blood, and from fresh organs of animals by extraction with amyl alcohol, and depended especially upon a positive Fröhde reaction as a test for the presence of the alkaloid. It is interesting to review some of the earlier medicolegal cases of morphine poisoning[24] and to learn on what ground experts came to the conclusion that this alkaloid was the toxic agent used. In 1871 Dr. Medlicott was convicted in Kansas of murder by morphine and atropine. An analysis of the cadaveric parts was made by the Stas method and affirmative results for morphine were obtained with the nitric, ferric chloride, Fröhde's, and iodic

acid tests. Atropine was determined by its reaction with sulphuric acid, and by physiologic tests. Dr. Kraus, of Tübingen, in 1878, reported the poisoning, in a German village, of a woman eighty-two years of age. The poison was administered in coffee, which the deceased drank, notwithstanding its bitter unpleasant taste. The symptoms were those of morphine poisoning, and death occurred within thirty-six hours. The analysis was limited to the application of the iodic acid test.

Another legal case was that tried in Portugal (1891-93). The defendant, Caso Urbino de Freitas, a physician, was accused of having poisoned three of his wife's nephews and of having caused the death of one of them by poison administered in enemas. The symptoms resembled those caused by opium in part only. The analysts claimed to have detected morphine, narcotine, and delphinine in the urine and in the viscera. They based their conclusion as to morphine on the insufficient evidence furnished by the iodic acid, Fröhde's reaction, and Lafon's reaction, the last one of which has recently been proved by Levine[25] to be characteristic not only of the morphine alkaloids but of all other compounds containing the phenolic group.

It is evident from the above citations that the qualitative methods used were far from sufficient to yield conclusive evidence as to the presence of morphine. In the case reported by Kraus and in the one reported by Wormley, neither the Fröhde reagent nor the iodic acid reagent is specific, for a positive reaction may be given by any number of organic substances that possess reducing ability. In the Medlicott case the nitric acid and the ferric chloride test could have been given by any number of phenols, while in the case of Urbino de Freitas, it has been very recently shown by one of us[25] that the Lafon test is a very reliable test for phenols in general. It can also be said that even a combination of tests for phenol and for reducing power is not any more specific, for organic compounds there are, other than the morphine alkaloids, which have the ability to reduce and which also give a positive reaction for the phenol group.

Since lack of specificity characterizes the color tests for the opium alkaloids it is not at all surprising to find that Vaughan,[26] in the Buchanan case in New York, pointed out that, while chemists for the prosecution swore to the presence of morphine and atropine in the dead body, all of the tests for the former made by the experts could be duplicated with the so-called putrefactive alkaloids. The six tests relied upon by the experts for the state were the ferric chloride, the nitric acid, the Husemann, the iodic acid, the Fröhde reaction, and the Pellagri reaction. The ferric chloride, the nitric acid reaction, and the Husemann reaction (sulphuric and nitric acids) are phenolic reactions. The Fröhde reaction and the iodic acid reaction indicate merely reducing ability. The only test that may at all be considered specific for morphine is the Pellagri test, which depends upon the conversion of the alkaloid into apomorphine. The test is believed to be specific but like other tests for morphine, it may be found to be non-specific when subjected to re-investigation. It must be remembered that in a putrefactive mixture of beef, Vaughan obtained a positive response with the Pellagri reaction, although no morphine was present. Rosenbloom and Mills,[27] however, claim that bacterial products formed

during aerobic and anaerobic putrefaction of certain human organs did not in any way give reactions simulating those due to morphine.

It is interesting to note that Vaughan[27a] has finally stated that the possible sources of error in mistaking putrefactive reactions for those of morphine have been definitely removed. This improvement has resulted from the greater purity of extractive reagents used and from better analytic methods. With reference to possible error due to putrefactive products, we may add in passing that van Itallie and Steenhauer have in 1925 reported that bases are formed by the action of bacteria. Some of these bases are precipitated by the usual alkaloidal reagents. Some of them even give color reactions resembling those of alkaloids. The investigators, van Itallie and Steenhauer,[27b] report that, in the toxicologic examination of a portion of liver from an exhumed cadaver, they isolated a compound which gave certain reactions for veratrine, but which proved to be the phenolic compound, p-hydroxyphenylethylamine.

In the light of our newer knowledge we must strengthen our evidence as to the presence of morphine in cadaveric material. It is very possible for a toxicologist to base his conclusion as to the presence of morphine upon five or six tests, every one of which represents but one characteristic property of this alkaloid, and which may also be possessed by non-alkaloids. It is for this reason that we strongly emphasize the fact that every toxicologic analysis for morphine should include at least one test from each of the six groups representing five distinct chemical properties and one biologic property characteristic of morphine and its associated alkaloids. The groups are given below.

Group I. Tests involving precipitation by the so-called alkaloidal reagents, which are general for all alkaloids.
- a. Phosphomolybdic acid.
- b. Iodine in potassium iodide.
- c. Potassium bismuth iodide.
- d. Potassium mercuric iodide.

With the last precipitating reagent Koller[28] devised a microchemical test depending upon the formation of yellow star-shaped or broom-shaped crystals or spherocrystals.

Group II. Tests involving the reduction of the reagents used.
- a. Iodic acid.
- b. Ferric chloride and potassium ferricyanide.
- c. Molybdic acid in concentrated sulphuric acid (Fröhde reagent).

Group III. Tests involving oxidation of the alkaloid.
- a. Nitric acid.
- b. Nitric and sulphuric acids (Husemann reaction).
- c. Iodic acid and ammonium hydroxide (Lefort reaction).

Group IV. Tests depending upon the presence of the phenol group.
- a. Selenious acid and sulphuric acid (Mecke reagent).
- b. Furfural and sulphuric acid (Udránsky reagent).
- c. Formaldehyde-sulphuric acid (Marquis reagent).
- d. Diazonium reaction (Lautenschlager reagent).
- e. Benzidine and concentrated sulphuric acid (Ekkert reagent).

Group V. Tests involving the preliminary formation of apomorphine.

a. Fuming hydrochloric acid, sulphuric acid, sodium bicarbonate, and tincture of iodine.

b. Sulphuric acid, sodium acetate and mercuric chloride (procedure of Grimbert and Leclère).

c. Concentrated sulphuric acid and concentrated hydrochloric acid (Pellagri reaction).

Group VI. Biological tests.

Straub-Herrmann reaction.

In addition to the chemical tests we should not overlook any biologic manifestations. In this connection the work of Straub and Herrmann[29] is very interesting. They have observed that white mice show a characteristic response to the injection of morphine or its salts. If even as little as 0.01 milligram of morphine is injected under the skin of the back of a white mouse of about 16 to 20 grams in weight, a certain definite picture is elicited. The back of the animal assumes a lordotic curvature; the hind legs give evidence of slight spastic paralysis; the tail assumes a characteristic S-shaped curvature, and finally falls over the back, with the tip lying over the ear. The whole behavior of the mouse reveals increased restlessness and stimulation. It springs into the air as a result of slight noises, especially those of high pitch. Other opium alkaloids—ethyl morphine or dionine, acetyl morphine or heroin, codeine, narcotine, narceine, thebaine, and papaverine—also respond to the Straub-Herrmann biologic test.

As in all clinical conditions, we should not disregard circumstantial evidence, such as the recent purchase of the poison, the finding of the drug or the container near the victim. Nor should we forget to ascertain from the attending physician or from those present the symptoms manifested, which may point to poisoning by the morphine alkaloids. On opening the stomach at autopsy, the presence of the characteristic odor of opium may lend corroboration to the chemical findings with reference to these alkaloids.

To make the condition more ideal for the detection of morphine or its allies it would be necessary to isolate the alkaloid in the form of crystals, which can be observed under the microscope. We are now investigating the possibility of utilizing the five chemical type reactions as microchemical procedures to be tried directly on one or more of the isolated crystals. We are also investigating the possibility of converting the morphine crystals into a derivative, like oxydimorphine, or into an acetyl derivative, like heroin, which may be identified by crystalline form, or in the case of relatively large quantities, by its melting point. Furthermore, it must be added that no toxicologic search for morphine is complete unless we make an attempt to isolate and identify its detoxication product, oxydimorphine, also known as pseudomorphine. This identification is possible by the use of a series of reactions proposed by Marmé.[30] Very recently Balls[31] has reported new methods of distinguishing pseudomorphine from morphine in a mixture of both compounds, and has described a quantitative procedure for their determination applicable to tissues.

SUMMARY

1. The formaldehyde-sulphuric acid reaction or the Marquis reaction is one of widespread applicability. It is given by all types of phenols, monohydric, dihydric, tihydric phenols and their various derivatives, phenolic glucosides and phenolic alkaloids. Nitrated phenols, however, do not give the reaction.

The reaction can be used for detecting phenols in biologic fluids. It can also be employed as one of the methods for testing the presence of phenol in the distillate containing volatile poisons obtained in a systematic toxicologic examination through steam distillation of suspected tissues.

2. The Marquis reaction is given by morphine and its allied alkaloids because of their phenolic character.

3. The color produced by phenols differs markedly from that given by the morphine alkaloids, although some phenols of biologic occurrence, such as p-cresol and pyrocatechol, give colors that are indistinguishable from those produced by the alkaloids.

4. In view of the fact that phenols may give the same color reaction or may modify the color reaction produced by the morphine alkaloids, caution should be exercised in the interpretation of the Marquis test as an indication of the presence of morphine or its derivatives.

5. Many tests known for the morphine alkaloids have been correlated and classified into six type reactions. It is suggested that clinical tests for these alkaloids should include at least one reaction taken from each of these six characteristic type reactions.

6. The study presented in the paper leads to the conviction that many of the analytical procedures for organic poisons, used in toxicologic examination and offered in legal evidence, are in dire need of thorough revision and critical re-evaluation. They were introduced into toxicology twenty-five to fifty years ago, when analytical methods in organic chemistry and in biochemistry were in their infancy. Where human life is at stake medicolegal safeguards should stand firmly on a basis of indisputable scientific facts.

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THE PHARMACOLOGY OF IRON AND ALUMINUM IN RELATION TO THERAPEUTIC USES*

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THE chemical relationships of iron and aluminum are so intimate, that in many cases they are difficult to separate. Because of these relationships one should expect their pharmacology to be much the same. In general this is true.

Under some conditions both iron and aluminum are toxic. A study and restatement of some of the less known, but important actions are interesting and may lessen the misuse of iron therapeutically.

Aluminum is the most abundant of metals, being present in the earth's crust to the extent of nearly 8 per cent. One is surprised, therefore, to find

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traces only in most forms of animal life. Because of its absence in animal life, an unwarranted toxicity has sometimes been attributed to it.

It is probable that aluminum like iron is essential for life, especially of plants, although in many cases traces only are found. Apparently it is more important in some plants than in others. Mazé believes that the presence of aluminum in the soil is necessary for the normal development of maize. Latshaw[1] found that the ash of maize contains 12.19 per cent aluminum and 9.74 per cent iron. Since practically all plants and all foods contain aluminum compounds either as essential ingredients or as contamination it is of importance to know the effect of aluminum when ingested by animals and man.

As is well known, iron is essential for the normal development of all green plants and perhaps all forms of animal life. The body of a man contains about 3.0 to 3.5 grams of iron, about 80 per cent of which is in the form of hemoglobin. Perhaps every cell in the body contains traces in an organic or nonionizable form. Aluminum is found, if at all, in traces only. Since iron is a normal constituent of the body and performs an important function, its administration is often looked upon as harmless, and generally beneficial, while the absence of aluminum and a widespread feeling against its use makes a comparison of the actions interesting.

It is not generally known that iron is a poisonous metal and for this reason is often harmfully used in therapeutic attempts. The toxicity of aluminum on the other hand has been grossly overestimated, due in part to the belief that it is a foreign element, and therefore necessarily toxic, and to misinterpretation of the work of Siem and Doellken.[2] These investigators gave aluminum parenterally and found it to be toxic. Since, when given by mouth, it is absorbed at most in traces only a very limited application can be made of their investigation. In addition, they used mainly aluminum tartrate, and tartrates are also actively toxic when given parenterally.[3] A restatement, therefore, is necessary.

IRON AND ALUMINUM IN COOKING UTENSILS

In 1913 the Lancet Laboratories[4] conducted experiments to see whether or not sufficient aluminum from aluminum dishes was dissolved to be objectionable. They cooked a great variety of substances in aluminum and iron utensils and came to the conclusion that there is no evidence to show that in ordinary cooking either the iron or aluminum is so strongly attacked as to produce objectionable amounts of soluble salts. All that could be found after the use of organic acids or salts in the cooking were the merest traces of either metal. They conclude, therefore, that either metal is suitable material for cooking vessels.[5]

The case is different when an alkali is present. Carbonate of soda has no action on iron but it attacks aluminum freely and it is well to exclude carbonate or bicarbonate from all aluminum cooking utensils. In this respect it should be mentioned that the heating of alkali in glass will dissolve the glass so that the management of aluminum cooking utensils would require the same ordinary application of common sense as is necessary in the case of other metals for a similar purpose.

ACTION OF IRON AND ALUMINUM SALTS ON ENZYMES

In 1904 I[6] found that the digestion of starch was inhibited by N/333 $FeCl_3$ while it required 3N/10 $AlCl_3$ or about 100 times as strong as the iron solution. It is possible that some correction in this should be made for hydrogen-ion concentration, which may not have been sufficiently considered at that time. This, however, would not alter the fact that in general iron is more toxic than aluminum.

ACTION OF IRON AND ALUMINUM SALTS ON THE DEVELOPMENT OF FISH EMBRYOS

Mathews[7] found that M/12000 $FeCl_3$ or M/20 $FeCl_2$ killed and prevented the development of fertilized fundulus eggs, while a concentration of M/9 $AlCl_3$ was required. This result indicates that aluminum chloride is only about one-half as toxic as ferrous chloride and over one thousand times less toxic than ferric chloride.

THE ACTION OF IRON AND ALUMINUM SALTS ON THE GERMINATION OF CLOVER SEEDLINGS

R. W. Ruprecht[8] compared the toxicity of iron and aluminum salts in the development of clover seedlings. His results show that ferrous sulphate exerts a toxic effect when it is present in over four parts per million. Aluminum sulphate was found to be toxic when present in the culture solution in concentrations greater than forty parts per million. According to this aluminum is ten times as toxic as ferrous sulphate which is much less toxic than ferrous salts. While these concentrations of aluminum prevent the germination of plants, more dilute solutions of aluminum have been found by Stoklasa[9] to be beneficial.

TOXICITY OF IRON AND ALUMINUM SALTS TO GOLDFISH

M/20 solutions of ferric chloride and aluminum chloride were prepared and a goldfish placed in each solution. The iron chloride solution killed the goldfish in an average of twenty-five minutes while in the aluminum chloride solution the fish lived approximately two hours.

ACTION ON FROGS

Hans Meyer and Francis Williams[10] investigated the toxicity of iron in 1880. They found that 5 to 10 mg. hypodermically in frogs caused a complete paralysis after twelve to twenty-four hours. I have found that iron is much more toxic than aluminum to frogs.

TOXIC ACTION ON DOGS

Kunkel[11] says that the deadly intravenous dose of iron is 20 to 50 mg. per kilo body weight while the toxic dose of aluminum is 150 to 300 mg. per kilo, or an average of 225. According to this, iron is six to eight times as toxic as aluminum. I have been able to confirm these figures. I found that the toxic dose for dogs when molecular solutions of the drug were injected intravenously was:

Ferric sulphate	30 mg. per kilo
Aluminum potassium sulphate	200 mg. per kilo
Aluminum chloride	234 mg. per kilo

Rabbits, cats and dogs were uniformly paralyzed and killed in twenty-four hours by doses of 30 to 60 mg. of iron per kilo intravenously.

Hans Meyer and Williams[12] found that 0.6 gm. of ferrous sulphate injected into the veins of a dog caused pronounced vomiting and diarrhea. Eight gm. given by stomach caused the death of a dog in twenty-six hours. Postmortem examination showed ecchymosis of stomach and intestines.

ACTION WHEN GIVEN BY MOUTH

Numerous feeding experiments on both men and animals have been performed with aluminum compounds and traces may be absorbed which apparently follow the routes of iron in the body.

Leary and Sheib[13] fed dogs and rats very large amounts and observed no ill effects, except possibly the death of one rat while receiving 300 mg. per day of aluminum. They conclude that their experiments have no special bearing on the question of aluminum compounds as used in the diet of man largely because the amounts used could not possibly obtain under any condition in man. With rats the ingestion of aluminum amounted to 300 to 3000 mg. daily per kilo of body weight.

ACTION ON MAN

The question of toxic action of aluminum in man was investigated by Chittenden, Taylor and Long,[14] who found that aluminum compounds furnished in foods up to 150 mg. per day for several months were without influence on health. No aluminum was found in the blood or urine. Winter Blyth[15] and his family used alum baking powder for months without any apparent injury.[15] Iron compounds, however, as used in medicine may exert a decidedly toxic effect.

Meyer and Williams[16] quote Frank as stating that 28 to 40 gm. of ferric acetate taken internally over a period of fourteen days caused great inclination to sleep, often colic and pains in stomach, vomiting and diarrhea. The blood was darker than normal and the heart beats were reduced to about one-half the normal.

LETHAL DOSE OF ALUMINUM AND IRON FOR MAN

The fatal dose of aluminum compounds is not definitely known. Blyth figures that the lethal dose of aluminum for a man of 68 kilos (150 pounds) is about 17 gm. or 3 ounces of ammonium alum. Death has been reported following the ingestion of 45 c.c. or 1½ ounces of the tincture of iron, which is equivalent to about 6 gm. or a dram and one-half of the salt.[17]

ACTION ON VITAMINES

It has been stated that metals may have an effect on vitamines, also that toxic agents may exert the action first on the germ cells. This is highly questionable and the reverse may be true. Recently Manville[18] has shown that vitamine B deficiency may manifest itself for the first time in the second

generation. If a metal had been given at this time it is probable that it would have been blamed for an effect on the germ-cells. Again, metals, and especially aluminum, have been found to exert the opposite effect. Daniels and Hutton[19] draw the following conclusions from their investigation on the mineral deficiencies of milk.

1. Rats fed exclusively on cow's milk seldom reproduce, and only a very small percentage of the young born survive.
2. The nutritive deficiency of milk appears to be due to the fact that milk in low concentration, namely, manganese, fluorine, and aluminum, tion of a new generation.
3. The addition to milk of those unusual mineral substances present in milk in low concentration, namely, manganese, fluorine, and aluminum, together with sodium silicate, has resulted in the production of five generations of normal young.

THERAPEUTIC CONSIDERATIONS

As aluminum is used chiefly externally and for its astringent properties there is little likelihood of its misuse. When taken by mouth in moderate amounts, there is little danger of toxic effect because traces only are absorbed. There is no excuse for its use hypodermically and it is only when used in this way that it is of toxic importance.

While iron also is used locally as a styptic, its main use is as a hematinic. In view of the toxic effects recorded, it should not be given hypodermically unless the conditions demand it, and such an occasion is rare. Most solutions of iron are precipitated at the site of action, hence are highly irritant. Deep muscular injection does not lessen this effect but merely hides it. If soluble salts which do not readily cause precipitation are injected they may be absorbed with sufficient rapidity to cause renal irritation or nephritis.

In view of the toxic actions cited, and since there is no adequate evidence that the hypodermic use of iron salts is advantageous, the hypodermic method of administration of iron must be looked upon as inadvisable and often harmful.

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LABORATORY METHODS

AN ACCESSORY TO THE CHAMBERS APPARATUS FOR THE ISOLATION OF SINGLE BACTERIAL CELLS*

By William H. Wright, Ph.D., and Elizabeth F. McCoy, M.S., Madison, Wis.

THE ever increasing interest in the variability and pleomorphism of microorganisms has shown the need for the study of cultures derived from single cells. Many forms of apparatus have been developed for the isolation of small objects with the aid of the microscope. The method of Barber,[1] first briefly described in 1904 and in detail in 1914, made use of very fine hair-like capillary pipettes. The pipettes were manipulated under a cover glass by means of the well-known Barber apparatus. The preparation of a series of very small droplets containing bacteria on the under side of a sterile cover glass, as used in this method, is still the best procedure for organisms as small as bacteria.

The difficulties encountered in controlling the best constructed forms of the Barber apparatus prevented its general use by many bacteriologists. This has been true of other methods, such as the one used by Topley, Barnard and Wilson.[2]

The greatly improved double micromanipulator of Chambers,[3] operating on an entirely different principle, is capable of easy and accurate adjustment.

The use of the single Chambers apparatus with the Barber moist chamber and technic for the isolation of single cell cultures of bacteria has been described by Kahn.[4] A single pipette is held in the micromanipulator, which is clamped on the side of the microscope stage. A moist chamber 19 mm. deep is used.

THE MODIFIED APPARATUS

A double form of the apparatus mounted on a heavy iron base with clamps for the microscope, as now made by the firm of Leitz, leaves little to be desired in the way of rigidity or precision of adjustment. We have used this type of the apparatus for several months for bacteriologic work.

The control of evaporation is important in the formation of the tiny droplets used in the isolations. The manufacturers supply very satisfactory moist chambers in three depths with cover glasses to fit. The shallowest moist chamber (10 mm.) lined with closely fitting strips of blotting paper on the sides has been found the most satisfactory. When saturated with distilled water these strips supply the moisture to the cover glass by evaporation

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and condensation. The shallow chamber has a small open end which checks excessive evaporation. It also permits the use of the ordinary substage condenser of the microscope.

For cell dissection and similar operations, there is an advantage in having the manipulator fixed in position in relation to the moist chamber and microscope. In bacteriologic work it is necessary to remove the bacterial cell

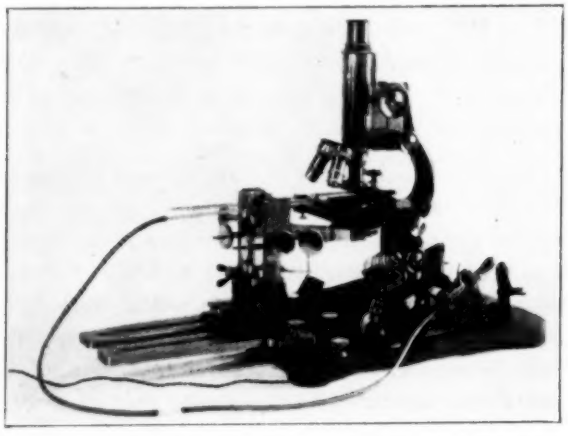

Fig. 1.—Modified form of the Chambers apparatus showing both of the micromanipulators in the working position.

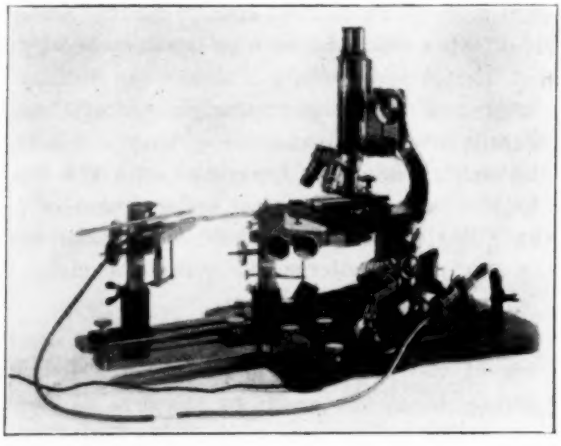

Fig. 2.—Modified form of the Chambers apparatus showing one micromanipulator in the distal position for removing pipette.

from the moist chamber after it is picked from a droplet. With the original double form of the apparatus mounted on a base, the pipette with the single cell must be removed by hand. The narrow opening in the moist chamber as well as the compact nature of the manipulators present difficulties which may result in the breaking of the delicate tip of the pipette or in contamination of the culture.

It seemed to us that the apparatus could be constructed so that the entire manipulator with its pipette could be moved in a straight line and in the same

horizontal plane away from the moist chamber. Plans and suggestions were submitted to the manufacturers and they have constructed extensions for the base along which the entire manipulators may be moved. The modified apparatus is shown in Fig. 1 with both manipulators in the working position, near the stage of the microscope. When it is desired to remove a pipette from the moist chamber the locking screw on the base is loosened and the entire manipulator is moved back on its slide to the position shown in Fig. 2. During the movement the pipette holds the same lateral and horizontal position it held while in the moist chamber. In this position it is a simple operation to remove the pipette or break off the tip in order to plant the culture.

The extensions for moving the manipulators away from the moist chamber also give the advantage of working with one apparatus without having the other one in the way. This allows much freer use of the vertical coarse adjustment than when both manipulators must be kept in the working position.

ISOLATION PROCEDURE WITH THE MODIFIED APPARATUS

1. Prepare a suspension of the organism or have a liquid culture ready.

2. Moisten the strips of filter paper in the sides of the moist chamber and place it in the mechanical stage of the microscope.

3. Clean a cover glass in cleaning solution and wipe dry with clean sterilized cheesecloth or gauze. We have found the most satisfactory cleaning solution to be one consisting of—

80 per cent ethyl alcohol	96 parts
Glacial acetic acid	3 parts
Ether	1 part

Greasing treatment recommended by some to make sure of isolated droplets is not necessary. Neither is heat sterilization necessary as one can always see any bacteria there may be in the condensation droplets. In hundreds of observations we have never seen contaminated droplets.

4. A small loopful of the liquid culture or suspension is placed a few millimeters from one end of the cover glass near the center.

5. The cover glass is placed over the moist chamber with the hanging drop near the closed end.

6. The clamps are now loosened and both manipulators slid all the way out. A small microscope lamp like the Leitz "Mignon" is centered on the mirror from the side and the light adjusted on the optical axis of the microscope. The heat from the lamp will help in the formation of the droplets. The rate of evaporation is readily controlled by varying the distance between the lamp and the mirror. In the 10 mm. moist chamber droplets 2 or 3 micra in diameter will hold an hour or more without evaporation. Usually droplets tend to become confluent only in the closed end of the moist chamber near the large drop, when the heat from the lamp is too great or the cover glass too clean.

7. It is of advantage to work with as low magnifications as possible on account of the size of the field and light intensity. The color screens of the

"Mignon" lamp are also very helpful in getting the best illumination. A 15 X hyperplan or periplan eyepiece allows much work to be done with a 16 mm. objective and a 4 mm. gives the greatest magnification ever necessary.

8. The under surface of the cover glass is now brought into focus. This is easily done on account of the small droplets in the field. By means of the mechanical stage the edge of the large drop is now brought part way into the field from the closed end of the moist chamber.

9. Pipettes as recommended by Chambers[3] should be sterile and ready. These are best sterilized in a metal case by use of hot air. Rigid shank pipettes with tips at 90° and 45° angles are the most satisfactory. A 45° pipette is used to isolate single cells in droplets and one of the 90° type to remove the cell from the moist chamber.

10. The 45° pipette is placed in the left hand manipulator which should be in the distal position, with the tip up and the pipette shaft horizontal. Being careful not to make the pipette tip strike the cover glass or sides of the

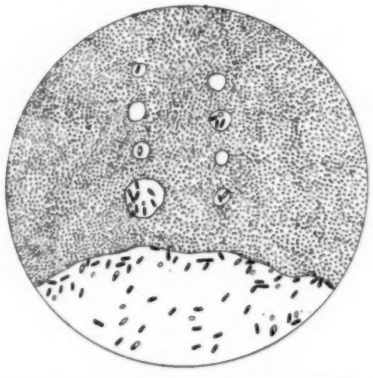

Fig. 3.—A microscopic field showing droplets outside of the large drop. The very small droplets without bacteria are formed by condensed water vapor.

moist chamber, the entire manipulator is carefully moved into the working position or until the tip of the pipette appears in the microscopic field on the side opposite the large drop. This can be most easily done with the lowest magnification; viz., with the 16 mm. objective or even with the eyepiece removed. Final lateral adjustments can be made with the manipulator screws when the eyepiece is replaced.

11. Vertical adjustment is easily made with the coarse adjustment. This adjustment may even be used for making droplets on the under side of the cover glass.

12. With the tip of the pipette nearly touching the cover glass so that it is clearly in focus, the large drop is moved forward until it strikes the tip of the micropipette. The pipette will immediately fill with liquid and organisms. With the vertical adjustment the pipette is slightly lowered and the cover glass moved back by means of the mechanical stage. The pipette is now raised until it touches the cover glass when some of the liquid with the microorganisms will be seen to wet the cover glass. Upon lowering the pipette

a small droplet will be left with several organisms in it. This operation can be repeated again and again until parallel rows of droplets have been made, such as shown in Fig. 3. The first droplets usually contain several cells. The last ones may contain one or two or none. The adjustments are so easily controlled that a single cell can be transferred from one droplet to another with ease. The droplets formed by condensation are often convenient for this purpose as they are sterile.

13. When the cell selected is isolated in a droplet, the manipulator carrying the 45° pipette is removed and the right hand manipulator with a 90° pipette is placed in position as before.

14. It is of advantage to have a length of small rubber tubing attached to the distal end of the 90° pipette and to a medicine dropper bulb, as shown in Figs. 1 and 2. If the bulb is slightly compressed at the time the tip of the pipette enters the droplet and then gently released, the droplet and the cell will be sure to enter the pipette. In case the operator cannot control the bulb in a satisfactory manner with the fingers, it can be done easily with the simple Hoffman screw clamp used by chemists.

15. By means of the mechanical stage the droplet and single cell are centered on the optical axis of the microscope. The 4 mm. objective is now focused on the droplet with the tip of the pipette immediately under it. With the vertical fine adjustment the pipette tip is made to enter the droplet and the pressure on the bulb released. The bacterium may be seen to enter the pipette tip.

16. The pipette is now lowered to a safe distance below the cover glass and the micromanipulator slid to the distal position as before.

17. The tip of the pipette carrying the single cell is now broken off with a fine pointed sterile forceps and dropped into sterile culture media. Some workers recommend breaking off the tip of the pipette by pressing it against the side of the test tube in the culture medium. We have not found this very satisfactory because the natural break includes the shank of the pipette which has been exposed during the whole operation. It might be contaminated.

DISCUSSION

The use of the improved apparatus as described is no more difficult than the usual routine operations of a bacteriologic laboratory and takes much less time to get pure cultures. We have made as many as six isolations in thirty minutes. Three per hour is an average number.

The securing of single cells is no longer difficult. The much more difficult problem is to get more of the single cells to grow. This difficulty has been encountered by all who have undertaken to grow cultures from single cells of bacteria. Our results have been much like those reported by other investigators. We have been able to get growth from about 75 per cent of single cell yeast cultures, 33 per cent of spore bearing aerobes and only about 2 per cent of spore bearing anaerobes. Single spore isolations have given much better results than those of vegetative cells.

The growth of isolated single cells of bacteria may involve a quantitative relation of the cell to the volume of the culture medium or a mutual action of several cells on each other. In connection with the first hypothesis Robertson[5] claims such to be the case for protozoa, although Cutler and Crump[6] have not obtained the same results. With bacteria it is difficult to understand why a vigorous cell, motile at the moment of transfer, will not grow in some of the same medium from which it was isolated.

CONCLUSIONS

The modified Chambers apparatus makes the isolation of single cell cultures of bacteria simple and practical.

2. The simplicity of the procedure is of great advantage for the direct isolation of pure cultures from mixtures.

3. The low percentage of single cell cultures that grow, as shown by the number of sterile subcultures, is ample proof that contamination rarely occurs. Such results also indicate the need for more study of the conditions which influence the growth of single cells.

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NOTE ON THE TUNGSTIC ACID PRECIPITATION OF BLOOD PROTEINS*

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IN THE preparation of protein-free filtrates by the Folin-Wu method the precipitation of proteins is frequently incomplete, so that correction of the reaction by the addition of extra sulphuric acid becomes necessary. Since in V. C. Myers' book on blood analysis this difficulty is repeatedly mentioned, we infer that it is not confined to this laboratory.

In our routine work we have adopted Haden's[1] very convenient modification which consists in incorporation of the sulphuric acid in the water used for laking the blood so that 8 volumes of 1/12 N sulphuric acid are used in

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the place of 7 volumes of water and 1 volume of 2/3 N sulphuric acid. This certainly has the advantages of a more rapid filtration and a higher yield of filtrate but, naturally, does not obviate the above-mentioned trouble. For this end we simply increased the concentration of the sulphuric acid above 1/12 normal. According to Merrill[2] the nitrogen content of blood filtrates is unaffected by changes in P_H between 4.6 and 1. In our own experiments, however, much lower acidity than that corresponding to $P_H = 1$ already disturbs the precipitation. Thus, employment of 1/8 N acid was found to cause considerable increase in the nitrogen content of the filtrate occasionally, and to give too low results in other instances.

The concentration of sulphuric acid we have ultimately chosen is 1/11 normal as against the 1/12 normal in the original Folin-Wu proportions. This concentration proved to be not excessive in cases where the original amount of acid, too, was sufficient for complete precipitation, and was adequate whenever incompleteness of the precipitation according to Folin and Wu entailed the addition of extra acid.

TABLE I

NO. OF SPECIMEN	1/12 N H_2SO_4		1/11 N H_2SO_4	
	NPN MG. %	B. S. MG. %	NPN MG. %	B. S. MG. %
266	36.2	169	35.7	168
268	28.6	101	30.5	106
305	30.2	84	28.7	87
306	40.8	87	37.8	86
313	33.7	—	34.7	—
315	34.4	—	34.2	—
325	29.6	117	31.4	110
326	30.0	87	32.2	86
327	36.4	98	35.6	90
329	31.0	—	30.0	—

Table I contains comparative determinations (picked at random as examples out of a greater number of experiments) of nonprotein nitrogen and sugar in filtrates obtained by the use of 1/12 and 1/11 normal sulphuric acid, respectively.

As can be readily seen the discrepancies are within the range of experimental errors.

As a result of our experiments the following procedure is recommended for the tungstic acid precipitation of blood proteins:

The blood is introduced into 8 volumes of 1/11 normal sulphuric acid, and after laking one volume of 10 per cent sodium tungstate is added. Shake, allow to stand for about five minutes, then filter.

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A COMPARISON OF THE QUANTITATIVE METHODS FOR THE BILIRUBIN OF THE BLOOD*

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EXPERIENCE within the last five years has demonstrated the diagnostic and prognostic value of the bilirubin content of the blood in the various types of jaundice. Although within the past eight years, considerable progress has been made in devising more exact technic for the bilirubin in blood serum, laboratory workers still are not agreed upon the most dependable and suitable method. Hence the results reported in the literature appear in variable terms and lack uniformity. Blankenhorn[1] in 1917 utilized a simple technic in estimating the bilirubin concentration of the blood serum. The blood serum was diluted to a point where the staining due to bilirubin was just perceptible in a column 1 cm. deep. Blood sera requiring a dilution of 20 or more gave a positive Gmelin's test. In some instances of marked jaundice, dilutions as high as 275 were obtained. In 1921 Meulengracht[2] introduced a more exact technic; 0.5 c.c. plasma were diluted in a graduated tube with physiologic salt solution until the color matched a standard in a similar tube. The standard was a 1:10,000 solution of potassium dichromate containing two drops of sulphuric acid per 500 c.c. Meulengracht stressed three possible sources of error, hemolysis, opalescence of lipemia and carotinemia. Normal blood plasma according to this method had a "plasma color" or "bilirubin number" of 1 to 5. In order to make the comparison with the standard more satisfactory, Gram suggested the use of blood serum instead of the plasma. Stetten[3] reported the practical application to surgical problems of the results obtained by Bernhard and Maue in their studies of the bilirubin of the blood serum. Bernhard and Maue utilized a standard similar to that of Meulengracht, but made their comparisons of the blood serum against this standard in a plunger type of colorimeter. The depth of the standard solution divided by the depth of the blood serum required to match it gave a figure which they called the icterus index. Stetten reported the average normal icterus index of 3.6, and found that it may vary from 1.6 to 13.5 without clinical icterus. The threshold figures varied from 8 to 14. Bernheim[4] has applied the icterus index determination to a study of the blood serum bilirubin in a variety of pathologic conditions. The normal range was found to be from 4 to 6, with a zone of latent jaundice extending from 6 to 16. Frank clinical jaundice was evident in all cases showing indices above 15.

We have in this modified Meulengracht test a simple direct means of measuring the intensity of color of the blood serum due to bilirubin. Bilirubin is not the only yellow-brown pigment of the serum. A part of the color of

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the blood serum is due to luteins or lipochromes, pigments extensively distributed throughout the animal and vegetable kingdoms. Meulengracht[5] reports that in the determination of the "plasma color" or "bilirubin number" these pigments introduce an error of 1, a quantity which can be neglected for all practical purposes. Carotin, a yellow-brown pigment found in the vegetable kingdom and in animal secretions appears isomeric and perhaps identical with some of the luteins. Carotin is found in human blood serum after the ingestion of meals containing vegetables. Hess and Myers[6] drew attention to the carotinemia in children on a diet of a high carotin content. These authors noted the skin of the subjects were pigmented to such a degree as to simulate a mild jaundice. However, the pigmentation was most evident on the palms of the hands, but the sclerae were not affected. The carotin also appeared in the urine. It is evident that the occurrence of a carotinemia introduces an error in the determination of the icterus index. For this reason principally, the icterus index of the blood serum has been considered by some authors as unreliable in estimating the bilirubin of the blood serum. Greene, Snell and Walters[7] state that the icterus index may be used satisfactorily in following changes in the degree of jaundice in patients with frank icterus. In such patients bilirubin is the preponderate coloring matter and changes in the serum color may reasonably be assumed to indicate variations in the amount of this pigment. Conditions are different in specimens of serum in which the bilirubin is little if any increased over the normal. In one case cited by these authors, the icterus index was 26, but the serum bilirubin was only 1.1 mg. per 100 c.c. (normal). The effect of a carotin-containing diet on the icterus index of the blood serum was studied by Bernheim.[8] Four normal subjects were given a meal containing carrots; three ate similar portions, and one ate a double portion. Three hours later, the icterus indices of the first three had been increased about 80 per cent above the control, while the fourth showed an index 250 per cent of his control. The following morning all of the indices had returned to normal. Errors due to carotinemia can easily be avoided by excluding from the diet all carotin-containing substances for twenty-four to forty-eight hours, and by drawing the blood after a night's fast. Obtaining the blood in the fasting state also obviates an unsatisfactory comparison due to postprandial lipemia. Bernheim and Meulengracht have emphasized the precautions necessary to prevent hemolysis. Preston[9] has described the use of a disc standardized against the dichromate standard, which has proved more satisfactory for comparison with blood sera, than has the dichromate solution. Whereas the intensity of the color of the solution fades on standing exposed to light, the color of the disc is permanent. The value of the icterus index of the blood serum in differential diagnosis in pathologic conditions involving liver function has been emphasized by Barrow, Armstrong and Olds.[10] After an extensive study of the clinical value of some recent tests of liver function Shattuck, Browne and Preston[11] reach the conclusion that the icterus index is the most useful single liver test that we have for clinical work.

Ehrlich and Proscher found that bilirubin enters into combination with an acid diazonium solution and van den Bergh utilized this reaction in a

quantitative method for bilirubin in the blood serum. The serum proteins are precipitated with alcohol and removed by centrifugation; the supernatant fluid is coupled with the diazonium solution and the color (reddish-violet) compared with a standard. The standard used was either an alkaline alcoholic solution of bilirubin with the diazonium solution, or an ethereal solution of ferric thiocyanate. Schamberg and Brown[12] have found the quantitative method proposed by van den Bergh a delicate index of the changes of bilirubin in the blood serum. These authors have emphasized the value of the results obtained by this method as guides in the treatment of patients with arsenicals. Thannhauser and Andersen[13] reported unsatisfactory results with this method because the unknown and standard did not match in the colorimeter. Moreover these authors have shown that the addition of the alcohol to the blood serum before the diazo reagents results in a loss of some bilirubin. Meulengracht has pointed out that precipitation of the serum proteins with alcohol introduces many errors. Part of the bilirubin is absorbed by the proteins and removed in centrifugation. A correction cannot be made for this loss because it varies greatly with different specimens. In some instances as in pernicious anemia, the loss was less than 5 per cent, but in cases of carcinoma of the pancreas, catarrhal jaundice and cholelithiasis the loss amounted to more than 50 per cent of the original concentration. In this connection it is interesting to note that Blankenhorn[14] observed that in cases of acholuric jaundice not all of the serum bilirubin was diffusible and this nondiffusible fraction did not pass into the urine. This retention and nondiffusibility of the pigment was due to a staining of the blood proteins by the pigment. The degree of staining varied with the concentration of the pigment and the length of time the plasma was exposed to the pigment.

Thannhauser and Andersen[15] proposed a quantitative method for the serum bilirubin based upon the coupling of bilirubin with the diazonium solution. Their method essentially differs from van den Bergh's in that the diazonium solution is added to the blood serum before precipitation of serum proteins. After coupling of the bilirubin with the diazonium solution has been completed (color change becomes stationary) the proteins are precipitated by ammonium sulphate and alcohol. After centrifugation the color in the supernatant fluid is compared against a standard. A stock standard of bilirubin in chloroform is maintained and from this the azobilirubin in alcohol is prepared for use in the colorimeter. Results are reported in units of bilirubin, one unit being equivalent to 1 part of bilirubin in 200,000 parts of serum. This procedure is theoretically ideal, but practically impossible, since pure bilirubin cannot be obtained on the market. Greene, Snell and Walters have advocated Thannhauser and Andersen's technic for the color development in the blood serum, but use van den Bergh's ethereal solution of ferric thiocyanate as a standard. These authors state that it is impossible to check their standard against pure bilirubin but assume that it is equivalent to 0.2 mg. of bilirubin per 100 c.c. Moreover it is admitted that the colors are not always identical, hence the comparisons are sometimes unsatisfactory. In 20 control cases without hepatic disease values from 0.3 to 1.4 mg. of bilirubin per 100 c.c. of serum were obtained.

TABLE I

PATIENT	DATE	ICTERUS INDEX	AZOBILIRUBIN MG. PER 100 C.C.	VAN DEN BERGH DIRECT	VAN DEN BERGH INDIRECT	CLINICAL JAUNDICE	DIAGNOSIS
1. J. M.	2– 3–26	3.3	0				Fibroma of uterus. 2ry anemia
2. E. B.	1– 8–26	6.0					
3. E. V.	2–19–26	6.0	pos.			0	Typhoid fever, acute cholecystitis
4. G. J.	3– 1–26	6.2	pos.			0	Chronic cholecystitis
S. B.	8–22–26	3.7	pos.			0	Chronic cholecystitis and appendicitis
5. L. S.	1–22–26	7.1	pos.			0	Myelogenous leucemia
6. P. C.	3– 1–26	7.1	pos.			0	Subacute endocarditis
2. H. A.	2– 9–26	4.7	pos.			0	
7. M. B.	2–23–26	8.3	pos.			0	Duodenal ulcer
8. M. R.	2–19–26	8.8	pos.			0	Diabetes and cholelithiasis
9. C. W.	3– 4–26	8.3	pos.			0	Diabetes and cholelithiasis
10. E. F.	3– 1–26	7.8	pos.			0	Pernicious anemia
11. M. W.	1–29–26	10.0	pos.			0	Banti's disease
12. S. D.	4– 8–26	10.0	pos.			0	After cholecystectomy
13. J. A.	4– 8–26	11.5	pos.			0	Chronic cardiovalvular disease
14. S. R.	4–23–26	10.7	pos.			0	Bronchopneumonia
15. A. S.	5–10–26	13.6	pos.			0	
16. E. S.	6– 3–26	15.5	pos.			0	Tabes dorsalis
	6– 7–26	11.5	pos.			0	
	6– 9–26	11.5	pos.			0	Acute cholecystitis
	6–15–26	10.0	pos.				
17. W. G.	1–12–26	11.5	pos.			0	
18. J. T.	4–28–26	13.6	pos.			0	
19. J. P.	2– 6–26	13.6	pos.			0	Hodgkin's disease
21. H. Y.	2–12–26	16.6	pos.			0	
22. A. M.	2–17–26	16.6	pos.			+	Splenomegaly and 2ry anemia
23. J. B.	5–10–26	22.5	pos.			+	Von Jaksch anemia
24. M. W.	1– 8–26	5.0	0.5			+	Luetic hepatitis
25. O. C.	1– 8–26	6.2	0.6			0	Angina pectoris
26. I. H.	6– 3–26	8.8	1.0			0	Mucous colitis
27. L. M.	1– 8–26	9.3	0.6			0	Pernicious anemia
30. D. P.	1–11–26	10.7	1.1			0	Pyloric ulcer, stenosis of right hepatic duct
28. A. S.	7– 9–26	10.0	2.8			0	Duodenal ulcer
						0	Chronic cardiovalvular disease. Cardiac decompensation
33. P. S.	3–15–26	12.5	1.4			0	Cholecystitis
36. R. O.	2–24–26	13.6	2.5			0	Pernicious anemia
31. B. P.	4–21–26	10.7	1.2			0	Duodenal ulcer

TABLE I—CONT'D

PATIENT	DATE	ICTERUS INDEX	AZOBILIRUBIN MG. PER 100 C.C.	VAN DEN BERGH DIRECT	VAN DEN BERGH INDIRECT	CLINICAL JAUNDICE	DIAGNOSIS
35. M. G.	8–16–26	12.5	1.8	−	+	±	Pernicious anemia
32. J. P.	8–23–26	10.7	2.1	−	+	0	Chronic myocarditis. Cardiac decompensation
37. A. T.	1– 8–26	13.6	2.9			0	Carcinoma of breast
34. E. C.	2–15–26	12.5	1.5	−	+	0	Chronic cardiovalvular disease
29. S. B.	2–19–26	10.0	1.1	−	+	0	
39. B. B.	2– 8–26	15.0	2.2	−	+	+	Catarrhal jaundice
	2–19–26	7.8	pos.	−	+	0	
38. J. T.	2– 3–26	13.0	1.7	−	+	+	Hodgkin's disease, enlarged liver
	2– 5–26	10.7	1.5	−	+	±	
42. E. K.	4–22–26	20.4	2.1	+	+	+	Lobar pneumonia
43. M. D.	8–22–26	20.5	3.5	+	+	+	Typhoid fever, cholecystitis
41. J. M.	8–26–26	20.0	2.7	−	+	0	Chronic cholecystitis
44. E. R.	8–11–26	22.1	3.5	−	+	+	Pernicious anemia
45. M. B.	5–17–26	23.0	4.0	+	+	+	Secondary malignancy of liver, primary not known
	5–20–26	20.4	2.5	+	+	+	
	5–28–26	20.4	2.5	+	+	+	
46. I. S.	6–28–26	24.9	3.4	−	+	+	Abdominal adhesions right upper quadrant
	6–30–26	17.6	2.4	−	+	+	
47. F. B.	2–26–26	26.7	4.2	−	+	++	Cholecystitis with adhesions
40. E. C.	1–20–26	18.7	1.8			+	Stricture of common duct after cholecystectomy
	2– 3–26	44.0	5.6			++	
	6– 5–26	11.0	pos.	−	+	0	
	6– 7–26	11.0	pos.	−	+	0	
	6–11–26	18.0	2.0	−	+	+	
48. A. D.	4–21–26	32.1	3.0	++	++	+	Postoperative sepsis. Hemolytic streptococcus
49. S. E.	12–29–26	35.7	3.4			++	Purpura hemorrhagica
	1– 8–26	15.0	1.5			+	
50. J. S.	3–18–26	40.8	4.4	++	++	++	Acute cholecystitis and cholelithiasis
51. C. P.	3–25–26	40.8	5.9	++	++	++	Splenomegaly. Cholelithiasis
	3–27–26	40.0	6.9	++	++	++	
52. J. S.	3–18–26	41.0	4.4			++	Chronic cholecystitis
53. M. C.	8–17–26	42.8	5.6	++	++	++	Catarrhal jaundice
	8–23–26	33.3	4.4	++	++		
54. M. L.	1–11–26	46.5	7.3	++	++	++	Cholecystitis and cholelithiasis
	2–24–26	37.2	3.9	+	++		
	3– 5–26	33.2	3.9	+	++		

TABLE I—CONT'D

PATIENT	DATE	ICTERUS INDEX	AZOBILIRUBIN MG. PER 100 C.C.	VAN DEN BERGH DIRECT	VAN DEN BERGH INDIRECT	CLINICAL JAUNDICE	DIAGNOSIS
55. E. K.	1- 8-26	50.0	7.1				Carcinoma of biliary tract, metastasis to liver
	1-13-26	99.6	12.4	++++	++++	++	
	1-28-26	90.0	12.4	++++	++++		
	2-11-26	130.0	13.1	++++	++++		
56. C. L.	2-26-26	60.0	9.5	+++	+++	++	Luetic hepatitis
	3-10-26	75.0	7.4	+++	+++		
57. A. M.	4- 9-26	75.0	9.3	+++	+++	++	Cholelithiasis
	4-17-26	37.5	3.6	+++	+++		Postoperative
58. G. K.	5-11-26	82.5	10.0	+++	+++	+++	Malignancy obstructing common duct
	5-21-26	81.7	9.2	+++	+++		
59. B. F.	6-16-26	86.7	15.8	+++	+++	+++	Cirrhosis of liver
60. M. S.	4- 3-26	91.6	10.1	+++	+++	++	Cholelithiasis
61. R. S.	7-12-26	92.3	9.3	+++	+++	++	Malignancy of biliary tract
	7-16-26	107.1	12.5	+++	+++		
62. J. B.	6- 3-26	95.0	11.7	+++	+++	++++	Carcinoma of pylorus. Mechanical obstruction of common duct
63. E. M.	4-28-26	105.0	17.3	+++	++++	+++	Catarrhal jaundice
	5- 3-26	142.0	19.1	+++	++++		
	5-10-26	56.0	7.9	+	+++		
	5-14-26	65.0	8.8	++	+++		
64. S. M.	3-13-26	110.0	13.8	++++	++++	++++	Malignancy of biliary tract
	3-20-26	149.0	17.2	++++	++++		
65. V. M.	7-26-26	112.0	14.0	++++	++++	++++	Carcinoma of stomach. Metastasis to liver. Complete obstruction
66. J. C.	6-21-26	136.0	20.2	++++	++++	++++	Carcinoma of pancreas
	7- 3-26	60.0	8.3	++++	++++	++	After cholecystogastrostomy
67. M. R.	3-24-26	150.0	21.0	++++	++++	++++	Malignancy obstructing common duct
	3-30-26	225.0	31.0	++++	++++		
	4- 5-26	165.0	25.0	++++	++++		
	4-16-26	216.0	27.0	++++	++++		
68. E. B.	8-11-26	166.6	29.6	++++	++++	++++	Chronic cholangitis. Stricture of common duct
	8-12-26	202.0	30.4	++++	++++		
	8-18-26	142.0	13.2	++++	++++		
69. V. S.	2-27-26	187.5	24.9	++++	++++	++++	Icterus gravis neonatorum

Since the appearance of this paper by Greene, Snell and Walters we have included in our studies of the bilirubinemia in cases of jaundice or with involvement of hepatic function, their modification of the van den Bergh method. In this communication we are presenting the results obtained for the icterus index, the qualitative and quantitative van den Bergh tests. The object of this study was to determine whether the results obtained by this more complicated van den Bergh technic warranted its adoption as a standard laboratory method for bilirubinemia in place of the icterus index. Parallel determinations of the bilirubin of the blood serum by these two methods have been made in 150 cases representing a wide range of hyperbilirubinemia. Of these 69 representative cases are reported in the table. All of the cases, except three (11, 14 and 54) were hospital patients. The blood was obtained after a night's fast and after a carotin-free diet for twenty-four hours. In the majority of instances the disc was used as a standard for the icterus index. For the ferric thiocyanate standard of the van den Bergh method, Merck's reagent quality ferric ammonium sulphate was recrystallized and dried in a desiccator over calcium chloride. The qualitative van den Bergh tests were separate procedures as described by McNee,[16] and not based upon color changes in the quantitative test as proposed by Greene, Snell and Walters.

In Table I are presented the findings for 69 cases, arranged in the order of their icterus indices. Of these 45 individuals had icterus indices varying from 3.3 to 24.9. The first 23 cases reported show indices from 3.3 to 22.5, but in these instances a satisfactory determination of the serum bilirubin could not be obtained by the quantitative van den Bergh method as described by Greene, Snell and Walters. In the first case no color was produced in the diazo reaction, but in the remaining tests the shade of color of the unknown varied to such a degree from the standard that a comparison in the colorimeter was impossible. In these instances the quantitative van den Bergh reaction is reported as positive in Table I, but an accurate estimate of the serum bilirubin could not be made. It is evident from an inspection of the table that 19 of these cases were within the zone of latent jaundice. It is particularly for cases in this stage of jaundice that a knowledge of the serum bilirubin is most essential. In the following 22 cases having icterus indices from 5.0 to 24.9 the color comparisons in the quantitative van den Bergh method were sufficiently satisfactory to warrant the calculation of azobilirubin in terms of mg. per 100 c.c. of serum. In many of these instances the color shades varied, but the readings could be confined to a small range on the colorimeter scale. The azobilirubin concentration in these sera varied from 0.5 to 4.0 mg. per 100 c.c. It is seen, however, that the figures for the icterus index and the azobilirubin do not run parallel. In Case 30, an icterus index of 10.7 was obtained with an azobilirubin of 1.1 mg. per 100 c.c., but in Case 28 the icterus index was but 10.0 and the azobilirubin 2.8 mg. per 100 c.c. In Case 39, when the icterus index was 15.0, the azobilirubin was found to be 2.2 mg., but eleven days later the icterus index had dropped to 7.8, and an accurate determination of the azobilirubin could not be made. Again in Case 40, when the serum bilirubin drops to within the limits of latent jaundice the azobilirubin determination becomes unsatisfactory.

A satisfactory color comparison of the unknown with the standard was made in all specimens of blood serum with icterus indices exceeding 25. However, in these cases the determinations of the azobilirubin do not parallel the icterus indices in changes in the bilirubin content of the blood serum. In the data reported by Snell, Greene and Rountree[17] on these tests for hepatic function in experimental obstructive jaundice, it is also seen that their figures for serum bilirubin by their quantitative technic do not parallel the "bile indices." The normal serum bilirubin content according to Greene, Snell and Walters is from 0.3 to 1.4 mg. per 100 c.c. In many instances in the cases studied by us (Cases 26, 27, 29, 30, 31, 33) normal figures were obtained for the serum bilirubin; the icterus indices, however, were definitely increased above normal. The increased icterus indices were accompanied by positive indirect van den Bergh reactions. Moreover in other instances with similar icterus indices, abnormal figures for the serum bilirubin were found. It is evident from the dates of analyses reported in the table that the unsatisfactory determinations of the serum bilirubin cannot be attributed to a lack of experience with the method. The chemical technic was beyond reproach, and the chemicals utilized were of the highest obtainable grade of purity. It is in those cases where the serum bilirubin lies between the range of normal concentration and frank clinical jaundice that the results of the quantitative van den Bergh method are least dependable. Bernheim also reports in her experience that the zone of latent jaundice cannot be accurately defined by this procedure.

We believe the sources of error are inherent in the method itself. At the present time it is not possible to check the standard against pure bilirubin. This fact alone opens the method to criticism, and the results obtained are of comparative value only. The figures no more represent the actual amount of bilirubin in the blood serum than does the icterus index. Ether is not an ideal solvent for the standard, particularly when a less volatile solvent, alcohol, is used for the unknown. Owing to the evaporation of ether in the colorimeter cup, the standard can be used for but one comparison. Moreover it is difficult to determine precisely when the coupling of the bilirubin with the diazonium solution has been completed. It was believed by the sponsors of this method that its use would obviate errors due to carotinemia and lipemia, but in our experience the occurrence of carotin or lipins in the blood serum influences the shade of color in the diazo reaction so that an accurate determination of the bilirubin cannot be made.

SUMMARY

In 150 cases representing varying degrees of jaundice parallel determinations of the serum bilirubin were made by the icterus index of the blood serum and the quantitative van den Bergh method as modified by Greene, Snell and Walters. When precautions were taken to avoid errors due to hemolysis, lipemia and carotinemia, the determination of the icterus index was found to be the more reliable measure of the serum bilirubin. The estimation of the serum bilirubin by the quantitative van den Bergh technic was unsatisfactory in about 50 per cent of the cases within the zone of latent jaundice. The

simplicity of the method recommends the icterus index as a standard laboratory procedure. The quantitative van den Bergh method cannot be standardized, and the technic is subject to many sources of error.

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THE PREPARATION OF COLLOIDAL GOLD SOLUTION*

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THE multiplicity of methods which have been described for the preparation of colloidal gold solution is, in itself, an indication that no one method is entirely satisfactory nor certain to produce good solutions with unvarying certainty.

The preparation of the solution, therefore, still remains the only part of the test presenting any difficulty.

Certain facts are clear from the many studies which have been made. It is evident, for example, that the laborious and time-consuming procedure originally considered essential by the earlier investigators is not only not necessary but even this, despite its careful precautions throughout every step, cannot always be relied upon to produce a satisfactory solution.

It is also known that good solutions must be absolutely neutral, acid solutions being too sensitive and alkaline solutions too insensitive to conform to the standards required, namely, no reaction with a normal spinal fluid, and complete reduction of 5 c.c. of solution by 1.7 c.c. of 1 per cent sodium chloride solution within one hour.

Undoubtedly, the predominant factor in producing unsatisfactory solutions is a lack of neutrality in the finished product which, regardless of the method used, may be due to a variety of causes such as the water used or varying degrees of acidity or alkalinity in the various reagents.

*From the Laboratories of The Atlantic City Hospital, Atlantic City, N. J.
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Many methods have been introduced to overcome this difficulty either during the preparation of the solution or by various measures after its completion.

A simple test of the reaction by means of alizarin as an indicator as commonly advocated is unsatisfactory because of the difficulty in reading the color change and it is more or less a common experience that solutions requiring extensive adjustment are apt to be of mediocre value.

For this reason other methods have achieved some vogue such as that described by Mellanby and Anwyl-Davies,[1] the modification of it described by Haden,[2] and that described by Novick,[3] the two former aiming to secure neutrality before, and the latter after the completion of the solution.

In our experience the Mellanby-Anwyl-Davies technic has always produced slightly acid and, hence, too sensitive solutions and the Haden modification solutions tending toward a slightly alkaline reaction.

Novick's titration measurement of the reaction is quite satisfactory but, while indicating the correction needed, does not obviate the necessity for, at times, marked readjustment of the solution tested.

It occurred to us, therefore, to combine the good features of all methods by which means the uniform and invariable preparation of good solutions is certain and a matter of relative simplicity.

The entire procedure follows:

Glassware: Must be free from scratches and thoroughly cleaned. We use Pyrex flasks which are cleaned in sulphuric acid-bichromate mixture followed by prolonged rinsing in flowing tap water and finally, in numerous changes of distilled water. Test tubes are similarly treated.

Reagents: All solutions are made with distilled water. Those required are:

N/20 Hydrochloric acid.
N/20 Potassium hydroxide.

One per cent gold chloride solution: Gold chloride, Merck, 15 grains; distilled water 100 c.c.

One per cent potassium oxalate: Potassium oxalate, C.P. *neutral*, 1 gram; distilled water 100 c.c.

One per cent potassium hydroxide: Potassium hydroxide, C.P. purified by alcohol, 1 gm. in 100 c.c. distilled water.

These solutions are stable.

Distilled Water: Double distillation is necessary, the second distillation being just prior to use. As noted by Haden, it is an advantage to add 1 c.c. of 10 per cent potassium permanganate and 1 c.c. of a saturated solution of barium hydroxide to each 2 liters of water just before the second distillation.

Preliminary Titration:

To each of six clean test tubes in a rack add 1 c.c. of 1 per cent gold chloride and add the following amounts of 1 per cent potassium hydroxide beginning with tube 1 and ending with tube 6: 0.6 c.c., 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., and 0.1 c.c.

A varying degree of turbidity will be noted in accordance with the degree of alkalinity, the most alkaline tube remaining clear.

Now add to each tube 1 c.c. of 1 per cent neutral potassium oxalate. Reduction begins at once. Complete reduction is indicated by a dense black precipitate, partial reduction by a lead color.

The tube containing the largest amount of alkali which can be added is that which shows complete reduction, usually tube 4 containing 0.3 c.c. of alkali.

Preparation of Solution:

To 100 c.c. of double distilled water in a Pyrex flask add 1 c.c. of 1 per cent neutral potassium oxalate and heat to boiling.

While this is heating, in a clean test tube place 1 c.c. of 1 per cent gold chloride and add the amount of 1 per cent potassium hydroxide solution indicated by the preliminary titration.

When the contents of the flask are boiling run in the gold chloride-alkali mixture drop by drop. The clear, red color develops at once when the solution is removed from the flame and allowed to cool.

The only objection to the method lies in the fact that it does not seem possible to prepare the solution in quantities greater than 100 c.c. at a time. As any number of solutions may be prepared quickly, added together, and corrected at once, as noted below, this objection is slight.

Final Titration and Correction:

When a sufficient volume of solution has been prepared, mixed, and allowed to cool, place 5 c.c. in a clean tube and add 1.7 c.c. of 1 per cent sodium chloride solution and set aside for one hour at room temperature.

If the solution is neutral and requires no correction, complete precipitation occurs rapidly; if alkaline, reduction is incomplete or absent in one hour.

Having thus determined in which direction correction is required, place eleven tubes in a rack and in each place 5 c.c. of the colloidal gold solution.

Now add, beginning with tube 1 and proceeding to tube 10, the following amounts of N/20 acid or alkali (as indicated by the saline tube just described): 0.05 c.c., 0.075 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.275 c.c., 0.3 c.c., 0.35 c.c., 0.375 c.c., and 0.4 c.c.

The eleventh tube is the control and receives no acid (or alkali).

Now add to all tubes 1.7 c.c. of 1 per cent sodium chloride and set aside for one hour at room temperature protected from light.

The tube showing complete precipitation and containing the least amount of acid (or alkali) shows the measure of correction required for 5 c.c. of solution from which the amount needed for the total volume of solution at hand is readily calculated.

(Acid or alkali solutions stronger than N/20 cannot be used as they affect the strength of the sodium chloride solution used as indicator.)

With the method outlined solutions can be prepared requiring a minimum adjustment of reaction and which will invariably give normal curves with normal fluids and paretic curves with paretic fluids—a sine qua non before any colloidal gold solution is put into use.

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A MODIFICATION OF THE ICTERUS INDEX*

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IN THE method commonly used for determining the color of the serum, the serum either diluted or undiluted, is matched against a standard solution of potassium dichromate or a glass standard. The resulting comparison is known as the icterus index. With the standard of 1 to 10,000 dichromate, which is used at present, normal blood with the colorimeter set at fifteen gives readings around three or four. This setting of the standard also gives a color which is rather pale for accurate matching. It is a well-known fact that the errors inherent in colorimetric work are accentuated when the two columns of fluid are not approximately of the same length.

It has seemed to us desirable, then, to modify the standard and setting so as to increase the strength of the color and to bring the columns to nearly equal lengths when used with normal bloods. Some of the standard can be poured into a graduated cylinder and the serum diluted to an approximate match in a similar cylinder. The standard can then be poured into the colorimeter cup and the factor of blood dilution taken care of in the calculation. This preliminary procedure is only necessary when the quantity of pigment is high.

We now make a solution of 3 to 10,000 dichromate (300 mg. per liter of water). This is preserved with a few drops of concentrated sulphuric acid in a dark bottle. The standard solution is set at 20 in the colorimeter and the reading made accordingly. To bring the results back into the terms now reported in the literature for the icterus index a slightly modified calculation is used which is given below.

$$\frac{20 \times 3 \times \text{number of dilutions}}{\text{Reading of unknown.}} = \text{Icterus index.}$$

*From the Department of Medicine, University of Maryland.
Received for publication, October 25, 1926.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PREGNANCY: Lactic Acid Content of the Blood During Pregnancy, Schultze, K. F. Zentralbl. f. Gynäk., July 3, 1926, l, 1759.

The average normal value was found to be 11 mg. per cent in nonpregnant women.

In thirty-seven pregnant women the average lactic acid content was 15 mg. per cent. There was some fluctuation. The author believes that lactic acid increases in the blood in the later months of pregnancy for reasons not clear.

In two cases of eclampsia the lactic acid content was enormously increased.

AGRANULOCYTOSIS: The Question of Agranulocytosis, Feer, W. Schweiz. med. Wchnschr., June 5, 1926, lvi, 551.

Feer contends that the condition first described in 1922 and since known as agranulocytosis is not an independent disease but a variety of septic disease representing an atypical sepsis and should be called "sepsis agranulocytotica."

BLOOD PRESSURE: Circulatory Tonics vs. Circulatory Depressants, Andrews, C. L. Jour. Am. Med. Assn., Sept. 18, 1926, lxxxvii, 928.

Andrews calls attention to the fact that many physicians and still more patients are focusing attention upon the blood pressure to the neglect of the underlying causative factors, and emphasizes that if such cases are properly classified the blood pressure will take care of itself. He emphasizes that:

There is abundant evidence that many treat hypertension as a disease itself.

There is a widespread fear that digitalis raises the blood pressure and should not be used in hypertension.

These cases can be partially classified by watching the blood pressure in conjunction with treatment results.

Tonic doses of digitalis should be given in hypertensive cases of long standing to support the heart muscle.

Patients with hypertension of long standing do better if the blood pressure is not lowered too much.

HYPERTENSION: Ultimate Results of Essential Hypertension, Paullin, J. E. Jour. Am. Med. Assn., Sept. 18, 1925, lxxxvii, 925.

In a review of seventy-six cases of essential hypertension observed from five to seventeen years, the number of cases was about equally divided between the two sexes.

The mortality for the group of men was 48.7 per cent, and for the women, 9.2 per cent, a difference in favor of the women of 39.5 per cent. During the five to seven year period of observation, the mortality was remarkably higher among the men.

Myocardial failure occurred earlier than cerebral hemorrhage among the men. It occurred much earlier in men than in women.

Death from cerebral hemorrhage in a majority of the cases was preceded by a previous apoplectic seizure. The greater number of deaths occur because of heart and blood vessel weakness.

The renal involvement in the late stages of this disease is usually very slight, only one death occurring in the series because of renal failure.

Essential hypertension occurring in women about the time of the menopause is relatively benign and is associated with fewer accidents and complications than for a similar group in men.

No definite conclusions can be drawn as to the end-result in a given case from a study of the blood pressure alone.

No definite prognosis can be given from a study of the blood pressure, the prognosis depending on the integrity of the heart and blood vessels.

NEPHRITIS: Experimental Production of Acute Glomerulonephritis: Use of Active Principle of Scarlatinal Streptococcus and a Consideration of Chronic Interstitial Changes: Preliminary Report, Duval, C. W., and Hibbard, R. J. Jour. Am. Med. Assn., Sept. 18, 1926, lxxxvii, 898.

The various types of acute glomerulonephritis, including the epithelial "crescent," endothelial proliferation, hyaline thrombi in the vessels of the glomeruli, hemorrhage into the capsular space, and complete necrosis of capillary tufts, can be produced experimentally in the rabbit under prescribed conditions, with the toxic principle of the scarlatinal streptococcus of the Dicks.

The experimental production of the nephritis here reported is of unusual significance, since it affords the opportunity to study the acute lesions in the order of their related sequence, and may form a means of tracing the changes that lead to a progressive diffuse nephritis. Furthermore, it opens a field of investigation that may lead to our better understanding of the causes and mode of production of certain forms of renal disease in man.

Streptococcal nephritis induced in the rabbit, and its complete analogy to the nephritic lesions of human scarlatina, is of especial interest since the causal excitant is in keeping with injurious substances more likely responsible for nephritis in man. While experimental nephritis has been produced with substances such as cantharides, snake venom and uranium, these are improbably excitants of the human disease and for this reason have not afforded an accurate basis for comparative study.

NEPHRITIS: Thyroid Therapy and Thyroid Tolerance in Chronic Nephritis, Epstein, A. A. Jour. Am. Med. Assn., Sept. 18, 1926, lxxxvii, 913.

The term chronic nephrosis is used to designate a group of cases in which a profound metabolic disturbance exists. To this disturbance the name "diabetes albuminuricus" has been applied.

The pathologic changes in the kidneys (tubular degeneration) are the consequence and not the cause of the metabolic disturbance.

The therapeutic requirements in chronic nephrosis are met in some cases by high protein feeding alone, and in others in conjunction with thyroid or thyroxin.

Cases of chronic nephrosis exhibit an unusual tolerance for thyroid and thyroxin.

The response to thyroid therapy is best measured by the cholesterol content of the blood. Thyrotoxic symptoms do not occur as long as a hypercholesterolemia exists.

Certain cases of chronic nephrosis are susceptible of complete cure by the intelligent and persistent use of high protein feeding and thyroid therapy. This may require a year or longer to accomplish.

MENINGITIS: Significant Chemical Changes in the Spinal Fluid in Meningitis, with Special Reference to Lactic Acid Content, Osnato, M., and Killian, J. A. Arch. Neurol. and Psychiatry, June, 1926, xv, 738.

The lactic acid content of normal spinal fluid during the fasting and resting state varies from 6 to 10 mg. per hundred c.c. The lactic acid concentration of the spinal fluid bears a close relation to its concentration in the blood. An increase of the lactic acid of the blood is associated with a similar increase in the spinal fluid, and the reverse of this appears true.

An increase of the lactic acid of the spinal fluid and of the blood was found in nephritis and epilepsy following the convulsions. In epilepsy the spinal fluid after the convulsions gave figures for lactic acid exceeding those for the blood obtained at the same time.

Spinal fluids obtained from cases of meningitis showed high figures for lactic acid. The source of this increased formation of the lactic acid appears to be the cellular metabolism. In some instances no decrease was noted in the sugar in fluids in which the lactic acid was increased above normal; in others, however, no reaction for sugar was obtained. In no instance did the increase in lactic acid account for all the sugar lost.

SCARLET FEVER: The Control of Scarlet Fever in Institutions, Colby, W. Jour. Am. Med. Assn., Sept. 18, 1926, lxxxvii, 919.

Positive Dick reactors can be immunized against scarlet fever streptococcus toxin.

Children under eight years of age may be safely given 3,000 skin test dose with this method.

In young children immunity is effected within as short a period as eight days, which makes possible the suppression of an epidemic by active immunization.

In older children, while immunity is established more slowly, repeated Dick tests at three months and six months indicate a marked progressive immunization.

On observing the possibility of mild scarlet fever developing in negative Dick reactors, it becomes evident that the strength of the test material should be increased.

PREGNANCY: Blood Pressure and Urinary Findings in 100 Cases of Normal Pregnancies, Faught, F. A. Jour. Obst. and Gynec., May, 1926, xi, 5.

There is practically no difference in the average blood pressure values in the primipara, as compared with the multipara.

There is slightly greater tendency for primiparae to show urinary abnormalities, which, however, does not appear to have any great significance.

We may expect to find a high incidence of albumin in the urine of pregnant patients, associated in many instances with casts and red blood cells.

The influence of these abnormalities on the average blood pressure findings is insignificant and well within recognized normal variations.

The persistent occurrence of albumin and other urinary abnormalities usually has little significance.

Individuals, not infrequently, show marked abnormal variations in systolic pressure, both below and above the normal limits, the occurrence of which does not necessarily indicate impending grave metabolic disturbances or toxic states.

The occurrence of glucose and indican as complicating factors during pregnancy must be taken into consideration since their incidence is comparatively frequent, and may be associated with comparatively great blood pressure abnormalities in the individual case.

Their significance and effect upon the pregnant woman is probably no greater than the other urinary abnormalities.

The mere elevation of systolic blood pressure does not indicate the approach of grave complications unless persistent, under which condition further light should be sought by a study of the blood for the detection of nitrogen retention and disturbance in the CO_2 combining power of the blood.

PREGNANCY: The Tendency to Acidosis in the Toxemia of Pregnancy, Levy, W. E. Surg., Gynec. and Obst., July, 1926, 38.

Eclampsia and preeclamptic toxemia are diseases of pregnancy manifested primarily by destruction of liver tissue. The liver is concerned with carbohydrate metabolism and storage. A deficiency of carbohydrates in the body leads to an imperfect combustion of the fats and in turn to the production of acetone bodies.

The author believes that he has definitely established that in the preeclamptic state and in eclampsia, an acidosis exists. Believing also that a distinct difference exists between preeclamptic toxemia and a toxemia due to a previous kidney disease, he has attempted to classify his cases as such.

Briefly he has included among those cases of preeclamptic toxemias such as show practically no renal involvement.

With the cases thus divided, the blood of the two types was next investigated along with the normal controls. As a result of the examination of the blood of fifty pre-eclamptics and eclamptics, it was found that there was a marked decrease in the blood-sugar content, a lowering of the carbon dioxide combining power, and practically no change in the nitrogenous constituents.

He concludes that the toxin of eclampsia produces definite destruction of the liver lobules.

The destruction of liver substances causes a derangement of the carbohydrate metabolism and glycogen storage.

The blood sugar and carbon dioxide combining power are lowered.

A state of acidosis is either imminent or present.

The rational treatment is with glucose or glucose and insulin.

He concludes that the outstanding chemical findings in the blood of eclamptic patients are:

1. A high uric acid.
2. A markedly increased lactic acid, not wholly due to muscular hyperactivity.
3. A decrease in the CO_2 combining power, which is very pronounced in certain cases.
4. A definite tendency towards a hyperglycemia, which is often associated with a high inorganic phosphorus.

There is usually no increase in the nonprotein nitrogen of the eclamptic blood. When such an increase is present, it may be associated either with a nephritis, upon which the eclampsia has been superimposed, or with the last stages of the disease. Furthermore, there is a slight, but definite decrease in the blood urea-nitrogen, as has already been pointed out by one of us.

It cannot be stated at present in how far the eclamptic blood picture is dependent upon the lesions usually observed in the liver.

TUBERCULIN: New Method for Tuberculin Test. A New Method of Dermo-Reaction and Its Clinical Value in the Examination of Adults, Ferrari, A. Rev. Med. Chir., Brazil, November, 1925, xxxiii, 627.

Ferrari makes an intracutaneous puncture and introduces tuberculin with a fine probe. The excess is removed after two minutes and the patient dismissed for five days.

When positive the reaction shows a reddish spot, distinctly visible, which may increase for several days, after which there is a slight desquamation and pruritus and finally a slight pigmentation lasting several weeks.

POLIOMYELITIS: An Outbreak of Poliomyelitis Apparently Milk Borne, Knapp, A. C., Godfrey, E. S., and Aycock, W. L. Jour. Am. Med. Assn., April, 28, 1926, lxxxvii, 635.

Detailed epidemiologic study apparently demonstrating that this disease can be spread by milk.

PERNICIOUS ANEMIA: The Common Picture of Sprue, Pernicious Anemia, and Combined Degeneration, Reed, A. C., and Wyckoff, H. A. Am. Jour. Trop. Med., May, 1926, vi, No. 3, p. 221.

The authors believe that a common clinical entity is embraced by the accepted diagnoses of tropical sprue, pernicious anemia, and subacute combined degeneration of the spinal cord. Review of published case records of these diseases and a study of our own cases point strongly to their being different intensities of manifestation of a common toxin. This toxin seems to attack the three systems; i.e., the blood, the gastrointestinal tract and the cord, to varying degrees although nearly always in cases fully studied, all three systems afford evidence of damage under any one of the three clinical diagnoses. For example, typical sprue may show evidence of cord changes and pernicious features in the blood. Typical Addisonian anemia shows evidence of sprue-like gastrointestinal changes and of cord degeneration. And finally, subacute combined degeneration of the cord is always associated with a progressive anemia which tends to become pernicious in character, and frequently with

achylia and other gastroenteric lesions. We suggest further that the toxin is more likely a group or type toxin than a unit chemical substance, and that its place of origin is in the digestive canal. Such a conception of these large disease groups means that sprue and pernicious anemia cannot be considered as unit diseases with a constant classical and characteristic type. But each is a group of variable clinical syndromes just as is the case in the group of what is called beriberi. This holds true to a lesser degree for combined degeneration.

Case reports are analyzed in the light of this conception.

POLIOMYELITIS: A Skin Reaction in Poliomyelitis, Rosenow, E. C. Jour. Infect. Dis., June, 1926, xxxviii, No. 6, p. 529.

Rosenow found that freshly isolated strains of the pleomorphic streptococcus, which produced flaccid paralysis in rabbits, when grown eighteen to twenty-four hours in pancreatic digest heart muscle broth to which one part in ten of ascites fluid was added, and the culture killed with phenol, 0.5 per cent, or tricresol, 0.3 per cent, yielded a useful toxic antigen.

The absence of marked reactions in persons fully recovered from poliomyelitis and who are known to be immune; the incidence of positive reactions inversely according to age, corresponding in general to the age incidence of poliomyelitis; the strongly positive reactions during the acute stage of the disease, and the negative reaction during convalescence, are considered as presumptive evidence that the test is a measure of susceptibility to poliomyelitis.

Numerous questions regarding the nature of the reaction have not yet been worked out. The immune serum prepared from horses with the pleomorphic streptococcus, and used with apparent benefit in the treatment of the early stages of poliomyelitis, has, however, a marked neutralizing power over the toxin, as determined by the skin reaction.

BACTERIOPHAGE: Bacteriophagy in Urinary Infection. Part I. The Incidence of Bacteriophage and of Bacillus Coli Susceptible to Dissolution by the Bacteriophage in Urines. Presentation of Cases of Renal Infection in Which Bacteriophage Was Used Therapeutically, Larkum, W. N. Jour. Bacteriol., September, 1926, xii, No. 3, p. 203.

Routine studies of urines from patients having urinary infections revealed the fact that bacteriophage was present in about 25 per cent of the urines while Bacillus coli susceptible to the action of bacteriophage was present in the same proportion of the specimens. The urines in which the susceptible colon bacilli were found were not necessarily the same as those in which bacteriophage was demonstrated. Normal urines, that is, urines not known to contain bacteria, were found to be free of bacteriophage.

When individual cases rather than urines were considered it was found that over 36 per cent of the cases studied had bacteriophage in one or more of the specimens of urine examined, while over 40 per cent (not necessarily including the above 36 per cent), were infected with a colon bacillus capable of being dissolved by a race of bacteriophage.

Almost without exception, the chronic cases provided urines in which only resistant bacteria were found, while the acute were seldom due to this type of colon bacillus. Bacteriophage too was found exclusively in the urines from individuals having acute infections.

The incidence of bacteriophage and susceptible colon bacilli in males and females was affected by the above condition. In practically every instance the males were suffering with chronic infections. Consequently the males, except in one case, were never a source of bacteriophage or susceptible bacteria.

Four patients subjected to treatment with the bacteriophage showed definite improvement after the treatment.

Bacteriophage is not found in rabbits' urine when bacteria of any type except the lysogenic strains are put into the bladder and maintained there for varying periods of time.

The introduction of colon bacilli into the body by the enteral or intravenous route fails to cause bacteriophage to appear in the bladder.

Damage to the bladder wall by means of hydrochloric acid does not result in the appearance of bacteriophage in the urine.

When introduced into the bladder, bacteriophage is eliminated within twenty-four to forty-eight hours.

As a result of these findings it is suggested that infection with lysogenic strains of Bacillus coli is alone responsible for the existence of bacteriophage in the urine.

Lysis of colon bacilli through the action of the bacteriophage can take place in the bladder.

Urine exercises an inhibitive action upon bacteriophagy.

Mucus, although apparently not affecting bacteriophagy, acts upon the colon bacilli in such a manner as to promote their removal from the bladder.

Surviving bladder tissue has no effect upon bacteriophagy.

Dead bladder tissue releases a principle resembling bacteriophage.

While it is impossible, on the basis of these experiments to state through what agency and to what extent modifications occur, it is obvious that bacteriophagy is not the same in the bladder as it is in the test tube.

PREGNANCY: Interagglutination of Maternal and Fetal Blood in the Late Toxemias of Pregnancy, Allen, W. M. Bull. Johns Hopkins Hosp., 1926, xxxviii, 217.

Allen investigated the iso-agglutination characteristics of 375 normal and 104 toxemic women and their infants.

He found no evidence that the late toxemias of pregnancy originate in iso-agglutination phenomena and believes previous reports based on too few cases.

URIC ACID: A Blood Urico-Oxidase and the True Value of the Blood Uric Acid, Flatow, A. Münch. med. Wchnschr., 1926, lxxiii, 12.

Flatow believes that the blood uric acid is much higher normally than appears from present methods of determination, due to the error introduced by a urico-oxidase derived from formed elements of the blood which is carried into the deproteinized filtrate, is active in weakly acid and alkaline solutions and is heat stable.

EPILEPSY: The Spinal Fluid in Epilepsy: A Study of Fifty Cases, Patterson, H., and Levy, P. Arch. Neurol. and Psychiatry, 1926, xv, 353.

No significant changes were found other than a great increase in pressure during an attack and the relatively frequent occurrence of colloidal gold curves similar to those seen in cerebrospinal syphilis.

NEOPLASMS: Mitotic Figures in Malignant Tumors as Affected by Time Before Fixation of Tissues, Evans, N. Arch. Path. and Lab. Med., June, 1926, i, No. 6, p. 894.

Evans has been accustomed to grade the malignancy of tumors in accordance with the number of mitotic figures present in sections which he reports in terms of the number per cubic millimeter of tissue.

A study was made of two tumors to determine the effect keeping tissue unfixed for varying periods. No material variation was found in the number of mitotic figures present.

PERNICIOUS ANEMIA: Treatment of Pernicious Anemia by a Special Diet, Minot, G. R., and Murphy, W. P. Jour. Am. Med. Assn., August 14, 1926, lxxxvii, 470.

The special diet used was made as palatable as possible and for each day was practically as follows:

From 120 to 240 gm., and even sometimes more, of cooked calf's or beef liver. An equal quantity of lamb's kidneys was substituted occasionally.

One hundred and twenty grams or more of beef or mutton muscle meat.

Not less than 300 gm. of vegetables containing from 1 to 10 per cent carbohydrate, especially lettuce and spinach.

From 250 to 500 gm. of fruit, especially peaches, apricots, strawberries, pineapple, oranges and grapefruit.

About 40 gm. of fat derived from butter and cream, allowed in order to make the food attractive. Animal fats and oils, however, were excluded as far as possible.

If desired, an egg and 240 gm. of milk.

In addition to the above mentioned foods, breads especially dry and crusty, potato, and cereals, in order to allow a total intake of between 2,000 and 3,000 calories composed usually of about 340 gm. of carbohydrate, 135 gm. of protein, and not more than 70 gm. of fat. Grossly sweet foods were not given but sugar allowed very sparingly.

This diet is rich in iron and purine derivatives containing about 0.03 gm. of the former and about 1 gm. of the latter.

Forty-five cases were thus treated with very encouraging results.

GINGIVITIS: The Chemotherapy of Gingivitis, Kolmer, J. A. Dental Cosmos, April, 1926.

Kolmer emphasizes that no one organism or group of organisms can be regarded as the primary or secondary cause of gingivitis. Some cases are predominantly bacterial, in others spirochetal forms which may occur in approximately normal mouths are responsible; in still others of the "trench mouth" type the fusiform bacilli and spirochetes of Vincent are the etiologic agents of importance. The E. buccalis may have some secondary importance as carriers of organisms or as opening up pathways for them.

The important feature of treatment is the correct application of the medicament so as to secure intimate and frequent contact with infected tissues together with the least disturbance possible so as not to hinder healing processes or extend the process by trauma.

Surgical removal of necrotic tissues, etc., is necessary.

The following solution is very effective:

Arsphenamine 0.3 gm.
Hot water 15 c.c.
Dissolve and add 15 c.c. of glycerine.

The solution is effective until oxidation has produced a blackish-green color.

A useful adjuvant is a tooth paste: mercurochrome or metaphen 0.5 gm. to 100 gm. of tooth paste. This is rubbed into the gums with the finger and after a minute or two brushed off. A lotion may also be used several times a day. The following are suggested:

Mercurochrome	0.1 gm.
Peppermint water	100 c.c.
or	
Metaphen	0.1 gm.
N/1 Sol. NaOH	4 c.c.
Peppermint water	96 c.c.

INSULIN: The Effect of Injections of Insulin and Dextrose on Blood Sugar, Thalhimer, W., Raine, F., Perry, M. C., and Buttles, J. Jour. Am. Med. Assn., August 7, 1926, lxxxvii, 391.

The intravenous injection of 10 per cent dextrose at a slow rate into normal persons induces a more rapid removal of sugar from the blood, so that during the latter part of the injection the blood-sugar level, instead of continuing to increase, actually declines.

Insulin mixed with the dextrose solution and given intravenously causes a more rapid and greater removal of sugar from the blood than when the insulin is given subcutaneously.

SMALLPOX: Smallpox without Eruption Following Blood Stream Inoculation, Blalock, J. B. Annals Clin. Med., March, 1926, iv, No. 9, p. 722.

The day after a transfusion in a case of pernicious anemia the donor presented a smallpox eruption. Ten days later the recipient complained of headache and an erythematous rash appeared on the thirteenth day, the temperature never rising above 100° F.

The course of the disease was uneventful. Vaccination on admission and on the third day after transfusion was unsuccessful.

The author concludes:

Inoculation of the blood of a person within the incubation period and within the period of prodromal symptoms of smallpox into the blood stream of another individual produced within the recognized incubation period the prodromal manifestations of smallpox, including the prodromal rash.

The organism or infecting agent of smallpox is present in the blood stream at least twenty-four hours before eruption.

The clinical syndrome referred to as "variola sine eruptione" may be produced following blood stream inoculation in a person partially protected by vaccination.

A striking example is furnished of the protective value of vaccination.

VAGINAL FLORA: The Vaginal Flora During Childhood and Puberty, Soeken, Gertrude. Ztschr. f. Kinderh. Feb. 20, 1926, xl, 727.

In childhood the vaginal flora is predominantly a coccus flora; but at the age of about eleven years this is, in most cases, replaced by a vaginal bacillus flora. This transformation is always connected with the presence of the signs of puberty. It takes place during an early stage of puberty, often long before the first menstruation occurs; and it is always a rapid and definitive change.

SCARLET FEVER: The Preparation and Clinical Application of Scarlet Fever Antitoxin, Anderson, J. F., and Leonard, G. F. Am. Jour. Med. Sc., September, 1926, clxxii, No. 6, p. 634.

A detailed and minute description of the methods used for the preparation of scarlet fever antitoxin. Because of its wealth of detail this paper cannot be abstracted satisfactorily short of transcription.

Analysis of the clinical results following the use of scarlet fever antitoxin prepared in a single laboratory in widely separated sections shows that the serum was specific for the various types of cases occurring in different sections of the United States.

The authors conclude that specific scarlet fever antitoxin may be prepared by the immunization of horses with filtered toxin.

Such antitoxin is specific against scarlet fever occurring in widely separated sections of the United States.

A properly prepared and standardized antitoxin is effective as a prophylactic when used in adequate doses.

When used for passive immunization, it should be given in not less than one-half of the average therapeutic dose.

A properly prepared and standardized scarlet fever antitoxin is effective in the treatment of scarlet fever, saving life and reducing the severity and frequency of complications.

GOITER: Histologic Changes Following Administration of Iodine in Exophthalmic Goiter, Giordano, A. S. Arch. Path. and Lab. Med., June, 1926, i, No. 6, p. 881.

From a study of glands taken at necropsy from exophthalmic goiter patients dying during crises there was found evidence that in most instances involution changes in the thyroid gland occur when iodine is administered to patients with exophthalmic goiter, and that, in general, the degree of involution of the thyroid parenchyma closely parallels the clinical course. The changes are similar in character to those described following ligation of the thyroid vessels, but they occur rather uniformly throughout the gland. It seems fair to assume that these changes are not characteristic of the method that induces them, for the author has also observed them in patients who came to operation during a period of remission of the clinical symptoms without any therapy other than rest. On the other hand, we have yet to explain the occurrence of marked involution changes in patients with definitely active true ophthalmic goiter. Such an occurrence is admittedly rare, but as yet no definite explanation has been given. This suggests that the anatomic picture does not always parallel the clinical course.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Diseases of the Heart**

THE second and third editions of this work contained few alterations from the first. Indeed, there were five printings of the third edition between 1913 and 1921 with practically no revision. The fourth edition, however, which was in proof at the time of the author's death has been quite extensively rewritten and brought up to date. The volume is a vehicle for the exposition of Sir James Mackenzie's theories of the physiology and pathology of the heart. As he has done in many of his more recent contributions, he points out the chief errors of the present system of medical research and the lack of finality of conclusions reached thereby, and points and leads the way to deeper and more thorough studies of fundamental principles.

"The methods of investigation pursued hitherto have led to the accumulation of a mass of symptoms and reactions. Most investigators end by adding to this mass and by introducing some new term. So confused is this mass that it is beyond the comprehension of any individual, and one result is that it effectually obscures the path of progress, so that the investigator himself raises a barrier to further progress.

"As all investigators are practically dealing with the same phenomena, and as the investigator in each branch sees the phenomena under different circumstances, each one applies a name which meets his own notion. The result is that the worker in one field is unable to understand the language of workers in other fields, although they are all dealing with the same kind of phenomena. There is thus lost that community of ideas and coordinate participation in work which is so essential to progress. The question arises, how can medical investigation be carried beyond this stage? Manifestly by understanding the factors concerned in the production of the symptom or reaction."

No instrument for the measurement of the functional efficiency of the heart or circulation now known will prove to be satisfactory in the opinion of the author.

"Before we employ any test we must know the nature of our measure. The employment of a measure as a measure without knowing what it measures gives a useless kind of knowledge or leads to fallacious results. Because a foot-rule can measure a yard of cloth, it does not follow that it can measure a pint of beer. Because an increased rate of pulse may indicate the sensitivity of the sinoauricular node, it does not follow that it can throw light upon the functional efficiency of the heart."

In his illustrations Mackenzie still uses polygraphic tracings in preference to electrocardiographic tracings because nearly as much information can be obtained therefrom and the investigator using the polygraph is studying natural phenomena which may later be studied without the aid of instruments, while the electrocardiologist is studying unknown forces. With the former the physician is studying movements which he can see and feel and must learn to recognize and to interpret. We know the forces that produce the various waves which are shown on a polygraphic record but it is not known what the agent is that produces the electro-

*Diseases of the Heart. By Sir James Mackenzie, F.R.S., M.D., F.R.C.P. LL.D. Ab. & Ed., F.R.C.P.I. (hon.) Cloth. Illustrated. Pp. 496. Humphrey Milford. Oxford University Press.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space alotted thereto.

cardiogram. There is no sign of importance exhibited by the heart which cannot be recognized by the unaided senses. It behooves the student to familiarize himself with the knowledge to be gained from instruments of precision but chiefly for the purpose of improving his own ability to dispense with these instruments.

All of the various affections of the heart are discussed in detail and interpreted in the light of Dr. Mackenzie's theories.

This volume is Mackenzie's last word on diseases of the heart in general and should be in the library of every cardiologist.

*Goiter: Nonsurgical Types and Treatment**

THE author includes under this designation simple endemic goiter, adolescent hyperplasia and the like, and Graves' disease or exophthalmic goiter.

The major portion of the work is devoted to a consideration of exophthalmic goiter. Bram takes a most emphatic stand against the consideration or treatment of this disease as surgical. He takes the stand that the basic pathology is probably not primarily in the thyroid gland but consists of an endocrine imbalance together with a disturbance in the vegetative nervous system, in which the thyroid is only incidentally playing a part. Whether this proposition be acceptable or not, his further argument against surgery is obviously logical. If, as has been suggested by the workers at the Mayo Clinic, the symptoms of Graves' disease are brought about by an incompletely iodized thyroxin molecule, a thyroid dysfunction rather than hyperfunction, our endeavor should be to provide the requisite amount of iodine or otherwise establish the manufacture of a normal thyroxin rather than merely to cut down the supply of the abnormal secretion. This is the logic for the administration of Lugol's solution.

Subtotal thyroidectomy for Graves' disease will naturally cut down, by the amount of thyroid tissue removed, the amount of abnormal thyroxin manufactured and to this degree will relieve the symptoms of the disease, but as the gland regenerates, increasing amounts of the abnormal substance will again be manufactured and the symptoms will often return. The author insists that the high incidence of recurrences after operation is an argument against surgery. On the other hand he considers toxic adenoma surgical for in this condition the diseased tissue is encapsulated and may be removed in its entirety. In Graves' disease removal of the entire gland would eventuate in death from myxedema.

The author's treatment is nonsurgical and includes rest, psychotherapy, readjustment, iodine and quinine medication, etc., removal of infectious foci, hygienic measures, and a high-calory low-protein diet. X-ray or radium treatment is taboo as producing very much the same effects as surgery, namely partial destruction of the glandular tissue.

The author gives comparative statistics which would indicate better results from medical than from surgical treatment.

The Surgery of Gastro-Duodenal Ulceration†

THE proper treatment of gastric and duodenal ulcer has long been a bone of contention between the surgeon and the gastroenterologist. Each claims superior results and presents statistics purported to demonstrate the inferiority of the medical and dietary treatment or the surgical end-results as the case may be. The recent trend is more toward conservative methods but there are still too many surgeons who insist upon the necessity for operation immediately the diagnosis has been made.

In this book we find a surgeon who, while writing on the surgical treatment of these diseases, prefaces his dissertation with the statement that with few exceptions medical treatment should always be given thorough preliminary trial. He insists on the fact known to all but

*Goiter: Nonsurgical Types and Treatment. By Israel Bram, M.D. Instructor in Clinical Medicine, Jefferson Medical College, Philadelphia, Pa. Cloth. Illustrated. Pp. 479. The Macmillan Company, 1924.

†The Surgery of Gastro-Duodenal Ulceration. By Charles A. Pannett, B.Sc., M.D., (Lond.) F.R.C.S. (Eng.) Professor of Surgery in the University of London, Surgeon to St. Mary's Hospital. Cloth. Illustrated. Pp. 154. Humphrey Milford, Oxford University Press.

some surgeons that ulcers undoubtedly heal under proper medical treatment, indeed not infrequently heal spontaneously with no treatment at all except that which nature imposes upon the sufferer by forcing him to rest and restrict his diet.

Those conditions which are most likely to necessitate surgical intervention are hemorrhage, perforation, organic obstruction and large callous ulcers which, though they may heal, do so with such devitalized and poorly nourished tissue that they are continually breaking down with the formation of new ulcers.

Dr. Pannett presents a critical comparative analysis of the various operations recommended and designates when each should be used. There are chapters devoted to perforation, hemorrhage, operative technic, and postoperative sequelae.

*Greene's Medical Diagnosis**

WHEN a volume has passed through its sixth edition it may be said to have established its own value. Greene's Medical Diagnosis is most ambitious in its scope and any who have been through it will agree that it approaches the realization of its ambitions. Both in size and utility it is comparable to French's Index of Differential Diagnoses. It differs from French in several respects, however. French is purely a reference manual while Greene is a combination textbook and reference instrument. Indeed, the volume may be best classified as a combined textbook on physical diagnosis and clinical pathology. The section devoted to the heart and vascular system is exceptionally good. Illustrations are bountiful throughout the book. Roentgen interpretation is incorporated under the various subjects and roentgenograms are abundant. A new section devoted to electrocardiography is profusely illustrated.

Marginal notations on all pages enable the hurried reader to see at a glance the general content of paragraphs in the text and thus facilitates the more rapid finding of the particular subject for which one may be looking. The index covers 160 pages and is most exhaustive. This is essential in a work of this type and adds greatly to the value of the book.

Nephritis†

THE difficulty of writing an authoritative treatise on nephritis which will still be up to date by the time it gets into print is obvious. While there has been no great addition to or change in our understanding of the functional pathology of nephritis since the publication of Cushny's last monograph, short contributions of the highest merit are continually appearing which deal with very closely limited phases of the physiology or pathology of the kidney or related conditions.

It is well that from time to time we should have a comprehensive review on the subject which will correlate the outstanding facts, monographs which in succession will mark periods of progress and will serve as stepping-stones for those interested in the disease under consideration, and enable the reader to avoid the alternative of wading through an enormous volume of individual contributions.

This function is satisfactorily fulfilled by Elwyn's volume on nephritis. The author has little to say of essential hypertension, classifying these cases rather as renal arteriosclerosis. He says that in these cases there is always a pronounced hyaline degeneration of the arterioles in the kidneys without similar changes elsewhere in the body.

He proposes a new solution of the eclampsia question. He discards the toxin theory and conceives of the process somewhat as follows: "With the beginning of pregnancy and continuous through it there is a gradual increase in the irritability of the entire neuromuscular mechanism which has to do with the function of uterine contraction. With the increase in the irritability of the neuromuscular mechanism, the rhythmic contractions of the uterus become stronger, finally terminating in the contractions of labor. This increased irritability is prob-

*Medical Diagnosis for the Student and Practitioner. By Charles Lyman Greene, M.D. Cloth. Illustrated. Pp. 1468. P. Blakiston's Son & Co., Philadelphia, Pa.

†Nephritis. By Herman Elwyn, M.D., Assistant Visiting Physician, Gouverneur Hospital, New York, N. Y. Cloth. Pp. 347. The Macmillan Company, 1926.

ably dependent upon a state of increased irritability of the presiding centers in the brain. The entire mechanism is placed on a higher plane of activity for the purpose of the final expulsion of the fetus.

"The close proximity of the centers for the vegetative functions in the brain permits the increased state of irritability to spread to the center for vasoconstriction in some cases. Impulses passing through the fibers of the thoracicolumbar outflow then cause the whole neuromuscular mechanism of the arterial system to become more irritable, and the arterial vessels to be in a state of greater tonic contraction. The irritability of the entire neuromuscular apparatus for vasoconstriction increases with the increase in the irritability of the neuromuscular apparatus for uterine contraction in the course of pregnancy. It becomes more marked at the time of labor. When the irritability is sufficiently high it causes arterial spastic contraction to a varying degree, slowly or suddenly, and initiates all the manifestations which we have considered the result of arterial spastic contraction."

*Diathermy with Special Reference to Pneumonia**

THIS is the second edition of a book previously reviewed in these columns. The author has added considerable material, particularly in the nature of case reports and has broadened the field of interest into more detailed consideration of the diathermy treatment of conditions other than pneumonia.

For those who are interested in the practical use of diathermy, the chapters on Diathermy Technic will be helpful.

Hay Fever and Asthma. A Handbook for the Patient†

TO JOSLIN in particular must go credit for the development of classwork with groups of sufferers from the same disease. After the diabetic instruction classes there came the nephritic classes and the asthma classes. Chandler Walker was, as far as the reviewer knows, the first to institute class instruction in asthma. A natural sequence to this has been the development of textbooks or manuals for the instruction of the patient himself so that he may have a better understanding of the nature of his infirmity and be more competent to himself apply the remedial measures prescribed by his physician.

Dr. Balyeat has written a very readable handbook on hay fever and asthma. In no way does it supplant the physician himself. Certainly, however, the reader after digesting its contents should make a better patient, a more co-operative one, and at the same time, a less impatient one.

The rationale of the new sensitization tests and treatment is made clear to the patient. The reviewer believes, however, that the author could safely have gone into greater detail in presenting in rather dogmatic fashion a layman's description of the immunologic principles involved.

In view of the large number of wheat sensitive individuals, the recipes for wheat substitute breads, ten in number, will be most welcome.

In his discussion of the nature of Kapok pillows, the author does not mention that these pillows are sometimes adulterated with small quantities of feathers. This, the reviewer has found in his own work to be a possible source of error of the greatest importance.

*Diathermy with Special Reference to Pneumonia. By Harry Eaton Stewart, M.D. Cloth. Illustrated. Pp. 228. Price $3.00. Paul B. Hoeber, Inc., New York. 1926.

†Hay Fever and Asthma. A Handbook for the Patient. By Ray M. Balyeat, A.M., M.D. Cloth. Illustrated. Pp. 198. Price $2.00. F. A. Davis Company, Publishers. 1926.

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EDITORIALS

Japanese Medical Education

IN THE fall of 1926 over 150 delegates visited Japan to attend the third Pan Pacific Science Congress in which the American medical world was so eminently represented by men like Dr. Victor C. Vaughan, and President Wilbur. During elaborate excursions the delegates were given an opportunity to make a somewhat hurried survey of Japanese scientific education and its accomplishments. What the writer saw at that time in the line of medical education is neither complete nor exact, but there were certain phases and tendencies in this work which were so strikingly different from most of ours that even a casual observer could not fail to take notice. These are recorded here at the suggestion of Dr. D. E. Jackson.

The "full-time clinical professorship" is an indoor sport of American medicine. Before our medical schools have yet experimented with this system in toto, there are many physicians in America who are thoroughly convinced that it is an absolute failure. Japan has practiced this system for

years, full-time men from departmental head to assistants in all colleges of medicine, nineteen in all. There are many collateral problems which are severely criticized by their own medical men. But there are several things which Japan has learned from the system. Among these are the following:

A college of medicine can be run in just the same way as a college of science or a college of arts. The men who teach in these colleges must be fundamentally investigators. The writer cannot name offhand a single example of a clinical professor in a medical college who does not hold a higher degree that was generally obtained by presenting a thesis based on some actual experimental scientific work. The most significant of all is the fact that those clinical men who hold the higher degrees are ranked as the highest of all clinical men in Japan. If we should ask a general practicing physician in Japan to name the first 100 most competent physicians and surgeons on the basis of clinical ability alone, the writer will venture to predict that at least 90 per cent of those selected would be men who have research degrees. It is assuredly true and eminently demonstrated in Japan that so-called "scientific medicine" does not prevent men from developing good clinical sense and judgment.

Not only are the clinical professors in Japan those who have in the past engaged in investigations of a fundamental and experimental nature but the contributions which continue to come from their own clinics are also of a thoroughly experimental character. One will be impressed with the titles of the articles contributed from these clinics. In a recent single number of a journal on experimental medicine having more than 500 pages, over 80 per cent of the articles were from a clinic on internal medicine in which were discussed such questions as internal secretions and gaseous exchanges of the blood and gaseous metabolism and blood flow to the brain under different conditions, the type of contributions one might well expect to come from physiologic or biologic laboratories. The recent monograph on intercellular oxidation and indophenol blue synthesis was written by the head of the department of internal medicine in a small medical college.

The conviction that research is the fundamental prerequisite for good clinical judgment and practice is so strong among the medical men in Japan that in most medical colleges there are at least 100 graduate students of medicine who are engaged in investigations in experimental medicine or in the preclinical sciences. These men are candidates for the higher degrees and are preparing for their careers by devoting their entire time to research. In one small medical college the writer counted at least fifteen doctors of medicine who were, as graduate students, engaged in research in the biochemical department alone. The chemical nature of placenta toxins, the metabolism of cholesterol, the action of cholin and its derivatives are a few of their problems that the writer happened to remember.

The fact that no graduate student from a college of science was found in a medical school is interesting, but of no significance, being entirely due to a different educational system, but the fact that so many young medical men are willing to spend three or four years in preparing for a higher degree by

doing pure research is exceedingly significant and should indicate the direction in which Japanese medicine is oriented.

Our own medical colleges take just pride in their physical equipment which is the universal envy of the world in general and which is a very important factor in the development of medical education. But how to distribute rightly this physical equipment and the financial resources of a medical college between pure research and routine instruction is a much debated question. It was a matter of much difficulty for the writer to obtain exact data as to the actual proportion of this allotment in the Japanese colleges. He does remember, however, one instance in a physiology department in which 80 per cent of the floor space was devoted to the research laboratories and in which, nevertheless, one of the most satisfactory courses in physiology for medical students is reputed to be given. When he saw three string galvanometers in one department, he could not help but admire the wisdom of the executive, when the price of such an instrument and the limitation of financial resources were considered.

Japanese medicine has much to learn. When one of their prominent professors of surgery told a medical academy that "The equipment and the general methods of Japanese medical education are not far behind America" he was probably overenthusiastic about Japan. But what he failed to emphasize was the *type of contributions* made by their clinical men to medicine.

—*Shiro Tashiro* (*D. E. J.*)

Some Clinical Tests for the Estimation of Circulatory-Respiratory Functional Efficiency

THE physiologic state, the so-called functional capacity, reserve or condition of the heart muscle, is generally accepted as the one great factor which practically alone determines the extent or limits of the physical activity of the individual at any time and, in a way, barring other disease processes, the span of one's useful existence. The problem of establishing this factor at all definitely is as difficult as it is important.

It is especially in the questionable or borderline cardiac cases, in which none of the reliable signs of heart disease are present, that one is usually desirous of obtaining some more satisfactory evidence of the integrity, the efficiency, resiliency, pliability or reserve of the circulatory-respiratory system. These cases sometimes present the symptoms of effort as dyspnea, palpitation, a labile pulse and blood pressure with a tendency to high levels on only slight exertion. Besides such cases of neurocirculatory asthenia which show no physical signs other than those of an irritable cardiovascular system, there are many individuals requiring further investigation, who have no complaints, but who have been found in periodical health surveys, employment applications or insurance examinations to have slightly suggestive signs, as systolic murmurs, changes in the character of heart sounds, deviations slightly beyond the established normal limits for blood pressure readings, or overactive hearts from the psychic effect of the examination. However, even in cases with reliable signs

of heart disease, but without evidences of congestive failure, it is often desirable from the prognostic standpoint to obtain accurate information of the status of the myocardium. The importance of the problem of devising a simple, reliable cardiovascular function test or tests is quite apparent.

Exercise tolerance tests with more or less crude standards of response in the rise of the pulse rate or blood pressure and the time necessary for return to the resting level have been in use for more than thirty years. These tests have their limitations, but are worthy of further consideration and standardization. The capacity for physical endurance is an objective criterion of the efficiency of the circulatory function and verifies and supplements the subjective opinion of the patient concerning his ability or limitation on exertion. The response to exercise often determines the management of the case, adding to the prognosis, as well as completing the cardiovascular study.

A system of *graduated tests* with the normal limits of response, such as have been outlined elsewhere,[1] is of value. The tests increase in severity and the system should be carried only so far as the patient's condition will safely permit.

The observations consist in preliminary studies of the heart rhythm, the respiratory and heart rate counted for a full minute and recorded while the patient is at rest in bed or sitting at ease in a chair. The systolic and diastolic blood pressures are taken and recorded.

After each of the tests that follow, records should be kept of the extent of rise in the pulse rate and that in the blood pressures, delayed, increase, no increase, or even a fall, and the time necessary for the return to the resting levels determined. The degree of breathlessness and exhaustion produced and the occurrence of precordial or cardiac pain, rhythm changes, murmurs or shifting of the apex impulse should be noted.

THE GRADUATED SYSTEM OF CARDIAC FUNCTION TESTS

1. Forced apnea (Russian). Determine the length of time in seconds that the patient is able to hold his breath. Note any changes in the heart rate or rhythm during the test. The normal length of forced apnea is from thirty to sixty seconds and there is an accompanying moderate slowing of the heart rate. This is a gross index of the vital capacity and should be compared with spirometer readings. In effort syndrome cases and cardiac failure the forced apneic test period is rarely greater than ten seconds.

Cooper advocated the determination of the respiratory ratio, that is the ratio of the length of time that the breath could be held after deep inspiration and after complete expiration. Normally, after deep inspiration the breath can be held forty to seventy seconds and after complete expiration twenty-five to thirty-five seconds. Variations from these ratios of 40/25 or 70/35 are suggestive of cardio-respiratory inefficiency.

Compression of the femoral arteries (Katzenstein). This test has been used to increase the load on the heart and determine cardiac efficiency by the response. The compression may be accomplished by pressure with the thumbs, a tourniquet or a blood pressure cuff. The method has found very few supporters.

2. Pulling of interlocked hands above the head for two minutes (Dock). In this test there is normally a slight rise of ten to twenty points in the heart rate with a drop to the resting level within a minute.

Herz has suggested as tests other muscular maneuvers such as have passive S movement against resistance, flexion and extension of the forearm, contraction of the muscles with or without extension of the lower limbs, and abduction and adduction of the thighs. These tests, however, have not been generally used.

3. Sitting up by the use of the abdominal muscles only and dropping back flat in bed five to ten times (Christian). This results in a rise of fifteen to thirty beats per minute and rise of as many millimeters of mercury in the blood pressure with a return to the resting level within two to four minutes. Mendelsohn's test consisted in observing similarly the effects of a succession of rapid changes from the vertical to the horizontal position. This test may be applied by having the patient bend forward ten times, attempting to touch the floor with his finger tips and coming back to the erect position with arms extended over the head.

4. Walking more or less briskly for one or two hundred feet on level ground (Schott), or on slight inclines (Oertel), has long been in use as a functional test for patients who were up and about. In order to provide for the accurate measurement of the amount of work done and its effect, Christ invented the steppage machine and attached a sphygmograph to the patient's wrist, recording the pulse rate graphically.

5. The staircase rapid ascent (Selig). Normally, walking briskly up a flight of forty steps causes an increase in the heart rate of twenty to thirty beats per minute with a prompt drop to the resting rate within one minute, while the blood pressure does not rise more than 10 mm. of mercury after this exertion. In mild cases of the effort syndrome, the heart rate will increase to 120 to 130 per minute and in severe cases to 150 or 160 per minute and the fall to normal exceeds two minutes in duration. The blood pressure likewise rises disproportionately. Reactions in excess of these figures are evidences of myocardial insufficiency. From the number of steps, the height of each, the incline and the individual's weight the amount of work can be calculated. Running up and down the staircase has been suggested to bring out latent weakness of the heart muscle and can be employed in only a very select group.

6. Hopping (Kahn). Twenty hops on each foot, raising the shoulder six inches each time, normally causes a rise of fifteen to twenty beats per minute in the heart rate and a rise of 5 to 10 mm. of mercury in the blood pressure. A drop to resting levels normally occurs within two minutes. There may be considerable variation if the patient's weight and his amount of co-operation are not taken into account.

7. Squatting (Stroud). With heels together and toes far apart, bending the knees as far as possible and coming back to the erect position fifteen times in a half minute's time will cause in a normal individual a rise of twenty-five to thirty heart beats per minute and a blood pressure increase of

10 to 15 mm. of mercury, with a return to within five points of the resting level after two minutes. Most normal subjects are moderately breathless after this test.

In selected cases the number of squats can be safely increased to twenty or thirty performed within a minute.

8. Stepping up (Schneider) on a chair twenty times, that is, placing one foot squarely upon a chair seat eighteen inches from the floor and raising the body to the full erect position, increases the heart rate in normal individuals twenty-five to forty beats per minute, with a rise in blood pressure of 10 to 15 mm. of mercury and a return to normal in two minutes. Practically all normal individuals show breathlessness after this test.

9. Lifting dumb-bells (Barringer), two fifteen pounders, from the floor to the full length of the arms above the head twenty times in forty seconds, causes in normal individuals a rise of forty to fifty heart beats per minute and an increase of 20 to 30 mm. of mercury in the blood pressure, which drops to within ten points of the resting levels within two minutes. Considerable breathlessness is provoked in a fourth of the normal individuals by this amount of work.

Lifting two twenty pound dumb-bells, through six feet, thirty times in sixty seconds, raises the heart rate fifty to sixty beats per minute and the blood pressure 20 mm. of mercury. Lifting the twenty pound dumb-bells sixty times in one hundred and twenty seconds increases the normal individual's heart rate sixty to eighty beats per minute and the blood pressure rises as much as 30 mm. of mercury.

Very often the effort syndrome case is unable to lift the twenty pound weights more than ten times and even with this amount of work his heart rate increases sixty to eighty beats and his blood pressure rises as much as 50 mm. and the drops within two minutes do not reach the resting levels by twenty to twenty-five points. Myocardial insufficiencies often show a delayed rise in blood pressure after these maneuvers.

10. Standardized work recording machines (Zuntz, Graupner, Wolffe). Ergostats or ergomometers on stationary bicycles or weight and pulley apparatus for automatically measuring the amount of work done in producing certain effects have been devised, but all have had definite limitations of practicability.

Wolffe[4] has recently advocated the use of a cardiovascular dynamometer built along the lines of a brake binder, which is rotated by the patient against the friction resistance by means of handles. The formula is derived and a work chart is drawn. The force in pounds is kept constant at ten to twelve for a given study and the number of revolutions is 50 to 100 with 3000 to 6000 foot pounds of work. The average rise in heart rate for 6000 foot pounds of work was thirty-five beats with a return to the resting level in four minutes.

THE CARDIO-RESPIRATORY TEST FOR CIRCULATORY EFFICIENCY

Abnormal variations in intrathoracic pressure have been found by Frost[2] to produce blood pressure reactions. These he considers to be definitely dependent upon the integrity of the cardiovascular system indicating the severity of

the strain and the efficiency with which it is resisted. An adaptable test has been devised in which a predetermined strain could be measured and the reaction recorded. A test such as this can be applied and months or years later reapplied under approximately the same conditions and at approximately the same degree of severity. This allows direct comparisons of reaction from time to time to determine the degree of progress of degenerative changes. The test is easily and safely applied to the aged and frail as well as to the young and sturdy, and permits observation of the response in the blood pressure, cardiac rate and rhythm during the application of the strain.

The apparatus* required is simple, compact, hygienic and easily portable in a small bag. The vital capacity readings with this type of windwheel spirometer are not absolute but relatively accurate and comparable. A blood pressure apparatus is also required. The gauge and spirometer are connected by means of a hard rubber Y tube and rubber tubing with a hard rubber stopcock inserted between the one branch of the Y tube and the spirometer while to the trunk of the Y tube is attached a piece of rubber tubing carrying the demountable sterilizable glass mouthpieces.

Of the nine steps or determinations, the first and ninth consist in control observations at the beginning and at the end of the test. In the second and fourth steps, increased intrathoracic pressure is produced by holding a full inspiration and by exhaling against the gauge. In the third and fifth steps decreased intrathoracic pressure is produced by holding a forced expiration and by inhaling against the gauge. In steps six, seven and eight increased intrathoracic pressure is produced and maintained through the greater part of the three exhalations and the approximate vital capacities are recorded by the spirometer.

TECHNIC, REACTIONS, INTERPRETATION

Step 1. Preliminary physical examination with especial reference to the blood vessels and the heart. The heart rhythm and rate are noted and the systolic and diastolic blood pressures are determined and recorded. It is advised to tuck the bowl of the stethoscope under the edge of the blood pressure cuff in order that the examiner's hands be free for manipulation of the apparatus. In practice only the systolic blood pressure in the significant part of each reaction is all that can be hoped for by one examiner.

Step 2. (Full inspiration held.) The systolic and diastolic pressure are taken and recorded and the subject is then instructed to inhale as deeply as possible and hold the inspired air in for ten seconds.

The air of a full inspiration should be retained by closing the glottis and allowing the chest and diaphragm to relax against the inflated lungs, the pressure of the relaxed walls producing the increased intrathoracic tension.

At the end of the inhalation the systolic, and if there is a second observer and apparatus, also the diastolic blood pressure are taken at least once but preferably twice, early and late, in the ten second apneic period.

*The outfit is furnished by the Taylor Instrument Company of Rochester, New York and consists of a Tycos vacuum-pressure gauge and a Simplex windwheel spirometer.

In normal individuals synchronous with this increase in intrathoracic pressure there is for two or three seconds, a quick initial rise averaging 5 mm. systolic pressure; then a rapid decline of 20 mm. or more, so that the systolic appeared to merge with the diastolic pressure, the level of which usually rises 10 to 15 mm. In the later stages of Step 2, as the systolic rises rapidly toward its resting level the diastolic pressure drops back toward its level. Both effects carry the levels a few points beyond the baselines as the air is released from the lungs.

The significant part of the reaction is considered to be the maximum decline of the systolic pressure. The systolic blood pressure fall should be at least 10 mm. of mercury and may drop in young adults until it merges with the diastolic. A failure to fall has been observed to occur in individuals with more or less rigid arterial systems and powerful hearts.

Step 3. (Full expiration held.) After a rest of ten seconds the systolic and diastolic pressures are again determined. The subject is instructed to exhale as far as possible and to refrain from inhaling for ten seconds.

The air should be excluded by closing the glottis and relaxing the chest and diaphragm against the deflated lung. The suction thus produced results in a condition of decreased intrathoracic tension.

At the end of the expiration the systolic and if there is a second observer and apparatus also the diastolic blood pressure are taken at least once but preferably twice, early and late in the second period just as inhalation is beginning.

In normal individuals synchronous with the decrease in intrathoracic pressure there is for about five seconds an initial decline in systolic pressure averaging 5 mm. of mercury, and then a gradual rise to 5 to 10 mm. above the resting level. In the later stages of Step 3 as the systolic rises the diastolic pressure falls 4 to 10 mm. which seems most characteristic of this step, and then gradually returns to normal. As inspiration is begun there is usually a quick increase in pressure up to 20 mm. above the original level, then a gradual decline to the baseline.

The significant part of the reaction is considered to be the maximum rise in the systolic pressure. The systolic blood pressure rise of more than 20 mm. of mercury above the resting level is taken to indicate an irritable unstable overacting, hyperactive cardiovascular system responding to strain with an excessive expenditure of energy.

Step 4. (40 mm. positive pressure held.) After a rest of ten seconds, the systolic and diastolic pressures are again determined. The subject is instructed to blow against the Tycos gauge, with the spirometer cut off by means of the stopcock, and to maintain a positive pressure of 40 mm. of mercury for about ten seconds.

The positive pressure is to be maintained by the chest and diaphragm rather than by the buccal muscles. A condition of increased intrathoracic pressure is produced similar to that in Step 2. The observations necessary,

the reaction, significant point and interpretation are similar to those given for Step 2.

Step 5. (25 mm. negative pressure held.) After a rest of ten seconds, the systolic and diastolic pressures are again determined. The subject is instructed to draw in against the gauge, the spirometer remaining cut off, maintaining a negative pressure of 25 mm. for about ten seconds.

The negative pressure is to be maintained by the chest and diaphragm rather than by the buccal muscles. A condition of decreased intrathoracic pressure is produced similar to that in Step 3. The observations necessary, reaction, significant point and interpretation are similar to those given for Step 3.

Steps 6, 7 and 8. (Expiration to full capacity through spirometer at 20 mm. positive pressure.) After ten second intervals the control blood pressures, systolic and diastolic are taken before each of these three similar maneuvers.

The stopcock in the tube to the spirometer is opened and the subject is instructed to inspire as deeply as possible and then to blow as long as possible through the spirometer. He must maintain a constant positive pressure of 20 mm. of mercury throughout by watching the gauge and keeping the indicator at the 20 mm. mark.

The blood pressure fluctuations are followed as in Step 2. The approximate vital capacity as indicated by the spirometer is recorded. The test is repeated twice in the same manner.

A condition of increased intrathoracic pressure is produced and maintained through the greater part of the expiration though of necessity gradually declining towards the end.

In normal individuals in the early stages of these tests reactions similar to those obtained in Steps 2 and 4, a quick initial rise followed by a rapid fall are noted. Later toward the end of expiration the systolic pressure began to rise, reaching 20 to 40 mm. above the resting level with frequently an added 5 to 10 mm. at the end just as inspiration began and then a rapid and later a gradual decline. The diastolic pressure followed as in Steps 2 and 4 rising at first and falling 5 to 10 mm. below the baseline just after the systolic peak was reached.

The significant part of the reaction is considered to be the maximum rise in systolic pressure. The systolic blood pressure should rise at least 20 mm. and at the most 50 mm. above the resting level. Failure to rise at least 20 mm. is considered an indication of a weakened myocardium or valvular obstruction; while a rise of more than 50 mm. is taken as an indication of an irritable, overactive cardiovascular system or an abnormally powerful heart.

Step 9. After about thirty seconds rest, the final observations are made. The systolic and diastolic blood pressures, the heart rate and rhythm, and the respiratory rate are noted. The systolic blood pressure is usually 5 to 10 mm. above the original level, the heart rate is as a rule increased 5 to 10 beats per minute, but frequently baseline or slightly lower final figures are obtained.

SUMMARY OF THE REACTION

Step 1. Resting Control.

2 and 4. Increased Intrathoracic Pressure.

Systolic B.P. fall at least 10 mm.

Failure to fall suggests arteriosclerosis. Diastolic B.P. rise 10 mm.

2 and 5. Decreased Intrathoracic Pressure.

Systolic B.P. drops 5 mm. at start then rises 5 to 10 mm. above the resting level. A rise of more than 20 mm. is taken to indicate an irritable overactive cardiovascular system. Diastolic B.P. fall 4-10 mm.

6, 7, and 8. Increased Intrathoracic Pressure.

Systolic B.P. as in 2 and 4 shows a quick initial rise followed by a rapid fall.

Later towards the end of expiration the systolic B.P. rises 20 to 40 above the baseline. A minimum of 20 mm. and a maximum of 50 mm. increase. Failure to rise 20 mm. is taken to indicate a weakened myocardium or valvular obstruction, while a rise of more than 50 mm. indicates an irritable, overactive heart. Diastolic B.P. shows a slight initial rise, then a fall 5-10 mm., then a rise. The base line fluctuation is only 5-10 mm. in normal individuals.

Step 9. Final Control.

COMMENTS

The cardiorespiratory test, as described by Frost, is certainly a most promising clinical method of estimating the functional state of the circulatory system. The many advantages of the test have been enumerated in the opening paragraphs.

A mechanical method of recording the blood pressure fluctuations continuously throughout the entire period of strain would simplify matters considerably and at the same time add an important graphic check. Considerable practice is required in the use of the manometer bulb and the release valve before one can follow accurately the rapid fluctuations in the blood pressure during the test. Frost says that after a little experience, however, the examiner knows intuitively the direction in which the pressure will fluctuate and will follow it more alertly. The taking of systolic pressures in the most characteristic and striking phase of the reaction is, however, all that one examiner can hope to accomplish accurately. The pressure must be released from the cuff after each test.

It is sometimes difficult to get a patient to understand the maneuver that he is to perform in Steps 2 and 3, but since the changes affected are accomplished by simpler subsequent procedures, there is adequate corroboration and

duplication. The method may bear even further simplification. Further studies with accompanying graphic methods, especially in cases with the reliable signs of heart disease, are necessary and are no doubt in progress in various laboratories. The results of these investigations will help to establish the final status of this clinical cardio-respiratory test.

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—*G. R. H.*